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Epidermal growth factor receptor: mechanisms of activation and signalling

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Abstract

The epidermal growth factor (EGF) receptor (EGFR) is one of four homologous transmembrane proteins that mediate the actions of a family of growth factors including EGF, transforming growth factor-α, and the neuregulins. We review the structure and function of the EGFR, from ligand binding to the initiation of intracellular signalling pathways that lead to changes in the biochemical state of the cell. The recent crystal structures of different domains from several members of the EGFR family have challenged our concepts of these processes.

Introduction

The epidermal growth factor receptor (EGFR) regulates the intracellular effects of ligands such as EGF and transforming growth factor-α (TGFα) [1–3]. For many years it has been known that upon ligand binding to the EGFR extracellular domains (collectively called the ectodomain), there is an increase in the proportion of dimerized receptor and the enzymatic activity of its intracellular tyrosine kinase domain increases greatly [4–6]. The EGFR kinase catalyses the transfer of the γ-phosphate of bound ATP to the tyrosine residues of exogenous substrates and the C-terminal domains of the EGFR, the latter in a *trans* manner [7,8]. After the induction of tyrosine phosphorylation, some signalling pathways appear to start with the recognition of the C-terminal phosphotyrosines by appropriate adaptor or signalling molecules [9,10]. The binding of ligand and activation of the EGFR kinase also induces the migration of EGFR from the caveolae/raft component of the cell membrane to the bulk membrane component [11] and the clustering of EGFR complexes into clathrin-coated pits that are subsequently internalized ([12–14]; see also Wiley et al., this issue) in a kinase-dependent manner [15,16]. Abnormal expression and/or mutation of the EGFR has been implicated in the progression of some classes of solid tumours (see Hynes article in this issue).

The EGFR also interacts with its three known homologues, ErbB2 (also called Neu or HER2), ErbB3 (HER3), and ErbB4 (HER4), in a ligand-dependent fashion to form heterodimers [3,17]. Differences in the C-terminal domains of these proteins results in changes to the repertoire of signalling molecules that interact with the heterodimers, thus leading to an expansion in the number of possible signalling pathways stimulated by a single ligand.

The strength and duration of intracellular signalling from the EGFR are also controlled by internalization and recycling of the receptor, which can be modulated by heterodimerization at the cell surface and by association with intracellular signalling molecules; these aspects of EGFR

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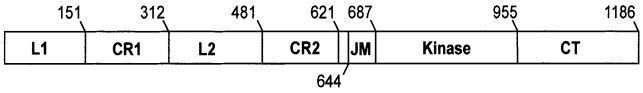

Fig. 1. Schematic representation of domains of the epidermal growth factor receptor sequence. The abbreviations used: L and CR, for the ligand-binding and the cysteine-rich domains [also known as I(L1), II(CR1), III(L2), and IV(CR2) or S1(CR1) and S2(CR1), where L and S refer to large and small]; JM and CT, juxtamembrane domain and carboxy-terminal terminus. The transmembrane domain (residues 622–644) is between the CR2 and the juxtamembrane domains.

behavior, relating to its trafficking in cells, are reviewed elsewhere in this issue (Wiley et al.).

The mechanism of the activation of the EGFR has been studied for many years; however, much remains to be determined. Significant progress has recently occurred; the crystal structures of extracellular portions of two ErbB family members and of the EGFR kinase domain have been reported [18–21]. Some of these structures reveal the modes of ligand binding, ectodomain dimerization, and the conformation of the apo-kinase domain. Full elucidation of the mechanisms of behaviour of both wild-type and oncogenic mutants of the EGFR should help with the design of new molecules to antagonize the action of the mutant or overexpressed receptor in cancer.

Architecture of the EGFR

The EGFR is synthesized from a 1210-residue polypeptide precursor; after cleavage of the N-terminal sequence, an 1186-residue protein is inserted into the cell membrane [22]. Over 20% of the receptor's 170-kDa mass is N-linked glycosylation and this is required for translocation of the EGFR to the cell surface and subsequent acquisition of function [23]; overexpression of the EGFR or altered glycosylation can reveal peptide epitopes suitable for antibody therapies [24]. The sequence can be categorized into a number of domains as shown in Fig. 1. The sequence identity of the EGFR family varies from 37% (53% similarity) for the EGFR and ErbB3 to 49% (64% similarity) for the EGFR and ErbB2. The amino acids identities can also vary significantly among the domains with the tyrosine kinase domains having the highest sequence identities (average 59–81% identity) and the carboxy-terminal domains having the lowest (average 12–30% identity). The three-dimensional folds of corresponding domains of the different EGFR homologues are expected to be similar with the possible exception of the heavily divergent C-terminal domains.

The EGFR extracellular portion (or ectodomain) consists of four domains that we refer to as the L1, CR1, L2, and CR2 domains (Fig. 1). The structure determinations of ectodomain fragments of the EGFR and ErbB3 show the L1 and L2 domains to consist of so-called β-solenoid or β-helix folds, which resemble the corresponding domains of the IGF-1 receptor [25]. Ligand binds between the L1 and L2 domains of the EGFR [18,19]. The orientation of the L1 and L2 domains of the unligated ErbB3 structure have been well defined and clearly must reorient to create the ligand-binding pocket. The CR1 and CR2 domains consist of a number of small modules, each appearing to be held together by one or two disulfide bonds. A large loop that protrudes from the back of the CR1 domain makes contact with the CR1 domain of the other receptor in the dimer [18,19].

The first module of the CR1 and CR2 domains contain conserved tryptophan residues (Trp 176 and Trp 492) that intercalate between the fourth and fifth helical turns of the β-helical L domain and sit in a hydrophobic environment that includes other conserved tryptophan residues (Trp 140 and Trp 453). An EGFR construct consisting of residues 1–476 lacks this second tryptophan interaction and does not bind ligand with high affinity [26]. Residues 557–617 in the CR2 domain are considered sufficient to target more than half of EGFR to the caveolae/raft component of the cell membrane prior to ligand binding [27].

The original assignment of the transmembrane domain as residues 622–644 was performed by visual analysis of the EGFR sequence [22], but other prediction methods indicate variation in the assignment of the boundaries of the transmembrane domains [28]. Nuclear magnetic resonance analysis of a peptide corresponding to the EGFR transmembrane and beginning of the cytoplasmic domain indicate that residues 626–647 are α-helical [28], suggesting that the transmembrane α-helix continues into the juxtamembrane domain. The juxtamembrane region appears to have a number of regulatory functions, i.e., downregulation and ligand-dependent internalization events [29], basolateral sorting of the EGFR in polarized cells [30], and association with proteins such as eps8 [31] and calmodulin (which is competitive with protein kinase C [PKC] association) [32,33].

The experimental three-dimensional structure of the EGFR kinase domain is similar to other tyrosine kinases [21]. By analogy with protein kinase structures, the ATP sits between the N-terminal lobe (dominated by a β-sheet) and the larger C-terminal lobe (mainly α-helical). The structure of the insulin receptor kinase with an ATP-analogue and small substrate peptide bound shows that the γ-phosphate group is positioned to be transferred to the acceptor tyrosine residue of the substrate [34].

The carboxy-terminal domain of the EGFR contains tyrosine residues where phosphorylation modulates EGFR-mediated signal transduction. There are also several serine/threonine residues (and another tyrosine residue) where phosphorylation has been inferred to be important for the receptor downregulation processes and sequences thought to be necessary for endocytosis. Residues 984–996 in the C-terminus have been identified as a binding site for actin [35] and may well be involved in the formation of higher order receptor oligomers and/or receptor clustering after ligand activation of the kinase domain.

A number of EGFR mutants have been observed in

Table 1
Mutations of the EGFR detected in tumour cells [36]; novel residues that occur at the splice sites are not shown[a]

Type	Alteration in sequence
EGFR vI	Translation starts at aa 543
EGFR vII	Deletion of aa 521–603
EGFR vIII	Deletion of aa 6–273
EGFR vIII/Δ12–13	Deletions of aa 6–273 and 409–520
EGFR vIV	Deletion of aa 959–1030
EGFR vV	Truncation at residue 958
EGFR.TDM/2–7	Tandem duplication of 6–273
EGFR.TDM/18–25	Tandem duplication of 664–1030
EGFR.TDM/18–26	Tandem duplication of 664–1014

[a] EGFR, epidermal growth factor receptor; aa, amino acid(s).

tumours where gene amplification has occurred (Table 1; reviewed by Kuan et al. [36]). The best characterized EGFR mutant is the Δ2–7 truncation (or vIII), in which amino acids encoded by exons 2–7 of the receptor (residues 6–273) are missing. This receptor mutant is constituently active and has defective downregulation behaviour [37]. Other EGFR mutants have deletions, regions of sequence duplication (summarized in Table 1) or defective kinase regulatory signals.

A soluble 105-kDa ectodomain fragment of the EGFR is produced by the A431 carcinoma cell line [38]. A secreted 80-kDa EGFR fragment that corresponds to part of the ectodomain of the full-length receptor has been observed to be produced in the placenta and in ovarian cancer [39,40]. Transcripts from other ectodomain fragments of human, chicken, and rat EGFRs have also been detected ([41] and references therein).

Ligand binding to the EGFR

The three-dimensional structures of the EGF- and TGFα-bound EGFR ectodomain fragments show that EGF and TGFα bind to the EGFR in the same mode [18,19]. Each bound ligand interacts with the L1 and L2 domains of a given EGFR molecule (Fig. 2). The conserved EGF residue Arg 41 (Arg 42 in TGFα) makes bidentate hydrogen bonds with Asp 355. Arg 41 is surrounded by Tyr 13 and Leu 15 (Phe 15 and Phe 17, respectively, in TGFα), orienting the arginine residue and shielding the salt bridge interaction from water molecules. Tyr 13 also interacts with Phe 357 of the receptor (Fig. 2A). The sidechain of Gln 384 of the EGFR makes two hydrogen bonds to the EGF mainchain atoms Gln43 O and Arg45 N (Glu 44 O and Ala 44 N, respectively, of TGFα) (Fig. 2A). The sidechain of Leu 47 (Leu 48 in TGFα) projects into a hydrophobic pocket consisting of Leu 382, Phe 412, and Ile 438 with the sidechain of Ala 415 at its base (Fig. 2A).

The EGFR L1 residues Gln 16 and Gly 18 contribute three mainchain-to-mainchain hydrogen bonds to Cys 31 and Cys 33 of EGF (Cys 32 and Cys 34 in TGFα), thus extending the larger of the two ligand β-sheets into the receptor (colored green in Fig. 2A). The sidechain of the EGFR residue Asn 12 also makes a hydrogen bond with the mainchain nitrogen atom of Gly 40 of TGFα [18] (Fig. 2A). The aliphatic sidechains of Ile 23 of EGF and Leu 24 of TGFα interact with the receptor sidechain of Leu 14 (Fig. 2A).

There are some compensating differences that distinguish the binding of individual ligands. A salt bridge between TGFα residue Glu 27 and Arg 125 of the receptor is not replicated in the EGF-bound receptor [18]) (Fig. 2B). The corresponding EGF residue is Leu 26, which sits in a similar position and interacts with Leu 14, Leu 69, Leu 98, and Ser 99 [19]. Clearly, both acidic or aliphatic residues can be accommodated in this ligand position, as is the case for the other known ligands of the EGFR [42,43].

Examination of the ligand-bound EGF and TGFα structures suggests that in addition to the conserved cysteine residues, Gly 18, Gly 39, and Tyr 38 (that can be replaced by Phe) are required to form or maintain the ligand conformation. Other ligand residues are also conserved or semi-conserved across the EGF family and support the notion that all ligands adopt the same folding and mode of binding as that for EGF and TGFα. EGF residue Arg 41 is completely conserved and its proximal residues Tyr 13 and Tyr 15 can be replaced with residues with aromatic and aromatic/aliphatic residues, respectively. Ile 23 of corresponds to aliphatic residues in all other ErbB ligands except for the weakly binding ligand, epigen [44]. EGFR residues Leu 14, Glu 355, and Phe 357 are conserved in all four of the ErbBs and residues Gln 384 and Asn 12 are conserved in ErbB3 and ErbB4. Conservation of these residues also supports the notion that ligands for ErbB3 and ErbB4 have the same mode of binding as that employed by EGF and TGFα to the EGFR.

The EGF residue Leu 47 is conserved among the ligands of the ErbB family except for amphiregulin, where the corresponding residue is methionine. The binding affinity of amphiregulin is several orders of magnitude less than that of EGF [45,46]. The predicted binding sites in ErbB3 and ErbB4 for the neuregulin residues that correspond to EGF residue Leu 47 are hydrophobic in nature, but appear to lack the defined pocket present in the EGFR due to the substitution of EGFR residue Ala 415 with Leu 412 and Leu 415 for ErbB3 and ErbB4, respectively. Interestingly, the β-forms of the neuregulins have an aliphatic residue equivalent to Leu 47 in EGF, whereas the corresponding residue for the neuregulin α-forms is proline [47,48]. As there are no other consistent sequence trends among the α- and β-forms of the neuregulins, the identity of this residue appears to be a major determinant of affinity.

The major ligand binding domain of the EGFR appears to be the L2 domain. The proteolytically generated fragment of the EGFR that contains the L2 domain and small regions of the CR1 and CR2 domains bind EGF and TGFα with submicromolar affinity [49,50]. Chimeras of the EGFR and

ErbB4 show that the L2 domain is the major binding determinant [51]. In contrast to the EGFR, the L1 domain of ErbB4 appears to confer the preference for NRG1β over EGF [51]. A proteolytically generated fragment of ErbB3 that binds NRG1β with 68 nM affinity consists of the L1 and most of the CR1 domain (residues 1–270). Ligand binding protects further cleavage at position 50 in the L1 domain [52]. The identity of the residues in the N-termini of the ErbB ligands' EGF domains appear to determine whether the ligand is able to bind to ErbB3 and ErbB4 [47,53–55]. The N-terminus of EGF and TGFα bind to the L1 domain of the EGFR [18,19]. Comparison of the chemical shift data from nuclear magnetic resonance experiments performed on the NRG1α EGF domain and an NRG1α chimera in which its N-terminus is substituted with that of TGFα shows that the chimera has altered sidechain packing in the region of the mutation, possibly rendering this chimera unable to bind to ErbB4 [54,56]. The contributions of the different regions of the ligand for binding to the EGFR, ErbB3, or ErbB4 accounts for the ability of betacellulin to bind to both the EGFR and the ErbB4 with high affinity [47,57].

EGF binds to both low affinity (K_D = 1–2 nM) and high affinity (K_D = 10–50 pM) sites on cells that express the EGFR [58]. The precise nature of the origin of the two affinities has yet to be determined; however, many studies have linked the apparent high affinity sites to the presence of receptor dimers [59–62]. Truncation of the long CR1 domain loop that mediates dimerization abolishes the apparent high affinity binding population [18]. Examination of the ligand-bound EGFR ectodomain dimer indicates that opening up the receptor binding pocket to release bound ligand is less likely to occur in the dimer than in the monomer. The high affinity binding of full-length EGFR on cells appears to be also modulated by interactions between its intracellular domains and other intracellular proteins or mutations of the tyrosine kinase domain [63–67]. The affinity of the EGFR is not necessarily a property of the receptor alone. It has been proposed that an intracellular protein mediates formation of the high affinity binding site of the EGFR [68,69].

The soluble ectodomain of the EGFR (residues 1–621) binds ligand and dimerizes to form a 2:2 complex [50,70,71]. The affinity for binding of EGF and TGFα to the soluble ectodomain of the EGFR is 100–500 nM ([50] and references therein). This affinity is comparable to the affinity of ligand binding to a proteolytically generated EGFR fragment that contains all of the L2 domain and only small portions of the surrounding CR1 and CR2 domains [49,50]. Surprisingly, removal of most of the CR2 domain from the EGFR 1–621, to produce EGFR 1–501, increases the binding affinity to 13–21 and 35–40 nM for EGF and TGFα, respectively [26]. A three-dimensional structure for the ErbB3 ectodomain reveals a conformation that excludes the possibility of ligand interacting with the L1 and L2 domains simultaneously [20]. High affinity binding (1–20 nM) has been detected for the EGFR ectodomain [71], but this is yet to be fully explained; while this could be due to a small proportion of preformed dimer, it is also possible that a small proportion of the receptor fails to form the "inactive conformation" seen in the crystal structure of ErbB3.

Ligand-induced EGFR oligomerization

The 2:2 ligand-EGFR complex forms on the cell surface [72]. Ligated EGFR ectodomain fragments undergo a novel mode of receptor dimerization [18,19]; a loop from the back of the CR1 domain from one receptor molecule interacts with a pocket at the base of the CR1 loop in the partner EGFR (Fig. 3). There are also some minor contacts between the CR1 loop and the L1 and L2 domains of the partner receptor. This interface participates in the formation of the physiological active dimer on the cell surface [18,19]. Key residues in the interface are conserved or substituted with residues that are expected to retain the dimer interactions (e.g., Phe $\rightarrow$ Tyr substitution where the phenol hydroxyl group is not used), indicating that the mode of binding is plausible for all of the ErbB proteins.

Superimposition of the L2 and CR2 domains from the ErbB3 ectodomain [20] onto the L2 and CR2 domains of the ligated sEGFR501 dimer indicates that the CR2 domains are likely to project to the same position of the cell surface (Fig. 3). The closeness of the C-terminal ends of the CR2 domains is consistent with ability to cross-link the receptors by the addition of a cysteine residue in the CR2 domain close to the transmembrane domain [61,73]. The positions of the superimposed CR2 domains are consistent with the concept that the long loops of the two CR2 domains (residues 572–582) in the EGFR dimer interact [74], but definitive evidence for this interaction has yet to be reported.

Localization of the EGFR to the cell membrane increases the effective concentration of the receptor, thus enhancing receptor dimerization relative to the soluble receptor ectodomain [50,75]. More than half of the unstimulated EGFRs on the cell surface are considered to be concentrated in caveolae, which account for approximately 5–10% of the membrane [11], thus further facilitating dimerization. A recombinant form of the EGFR, consisting of only the transmembrane and kinase domains, is capable of self-association [76]; thus, the transmembrane and kinase domains have active roles in stabilizing the dimer. Indeed the presence of the transmembrane domain enhances ligand-induced dimer formation in solution [77].

The formation of heterodimers of the ErbB family in solution is less well characterized than the formation of the EGFR homodimer. Most notably, the stoichiometry of the ligands and receptors in the heterodimer complexes is unknown. ErbB2 is the preferred interacting partner for the EGFR [78,79]. This interaction has been reported to reduce the rate of EGFR degradation [80]. It has been suggested that so-called heterodimers may actually be heterotetramers,

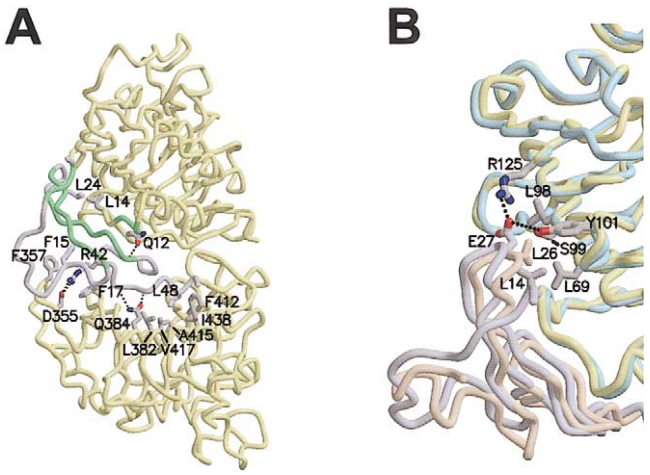

Fig. 2. Interactions between the epidermal growth factor (EGF) receptor (yellow) L1 and L2 domains with bound transforming growth factor-α (TGFα) (metallic blue). Residues that contribute to a β-sheet that involves both the receptor and the ligand are colored green. The sidechains of selected ligand and receptor residues are shown as sticks. Selected hydrogen bonds are represented as dotted lines and mainchain atoms involved in these interactions are not rendered. (A) C_α worm representation of TGFα bound to the EGF receptor residue 1–501 [18] with a number of key interacting residues displayed. Carbon atoms of the sidechains of the EGF receptor are colored grey to increase their visibility. (B) Detail of interaction between TGFα residue Glu 27 with the EGF receptor L1 domain. For comparison, the EGF receptor L1 domain (colored cyan) and EGF (dark pink) [19] are shown as superimposed on the TGFα-bound EGF receptor structure. The sidechain of EGF residue corresponding to TGFα Glu 27, Leu 26, is also rendered to illustrate how this residue can be accommodated by the receptor. This figure and Fig. 3 were created by using the programs Molscript [292] and Raster3D [293].

possibly organized around a nucleating ErbB homodimer [81,82]. The formation of secondary hetero-oligomers can be induced by a ligand for a third ErbB protein. For example, EGF stimulates the formation of ErbB2–ErbB3 hetero-oligomers in cells that also express the EGFR [81,83]. Johannessen et al. [84] have reported constitutive EGFR-ErbB2 association, although exposure to EGF increased the phosphorylation of the ErbB2 residue Tyr 1248. Hetero-oligomers involving the EGFR and cell surface receptors outside of the ErbB family, such as complexes involving the EGFR and the platelet-derived growth factor (PDGF) receptor, have also been reported [85]. Such heterocomplexes may be mediated by interactions with intracellular adaptors and/or scaffolding systems [68].

Ligand-induced activation of the EGFR

In the absence of ligand binding, the EGFR exists on cells as both monomers and dimers [72,73,86,87]. Yet ligand binding to the EGFR kinase is required to elevate the receptor's tyrosine kinase activity. The position-dependent effects of adding a cysteine residue in the membrane-proximal part of the EGFR's extracellular region suggests that a ligand-associated orientation of the EGF dimer is required for activation of the tyrosine kinase domains [73]. Clearly, dimerization of the EGFR, while necessary, is not sufficient to activate the intracellular kinase.

Moriki et al. [73] demonstrated that it was possible to form cross-linked dimers of the EGFR by adding cysteine-containing insertions of nine residues to the membrane-proximal region of the EGFR (at position 618). These EGFR dimers form in both the absence and the presence of ligand. There are position-dependent preferences for formation of dimers cross-linked by these cysteine residues. The results of this study are consistent with a previously proposed model, referred to as the rotation-twist model, in which ligand-binding induces the predimerized EGFR to twist about a pivot point near or in the transmembrane domain and reorients the intracellular domains to form an active kinase configuration [73,86]. At present, no other regions of the ectodomain have been directly implicated to control receptor reorientation on ligand binding.

Comparison of the structures of the first three domains of the EGFR and ErbB3 show that significant rearrangements of the ectodomain can occur as changes in the CR1 domain, altering the relative positions of the L1 and L2 domains from those of the ligand-bound structure [18–20]. Most notably, a change of the conformation of the C-terminal end of the CR1 domain alters the juxtaposition of the L2 domain with respect to the preceding two domains. From Fig. 3, it can be envisaged that changing the angle between the CR1 and L2 domains, while conserving the CR1-CR1 interface, completely alters the L1–L2 juxtaposition and consequently the juxtaposition of the succeeding domains. Thus, the role

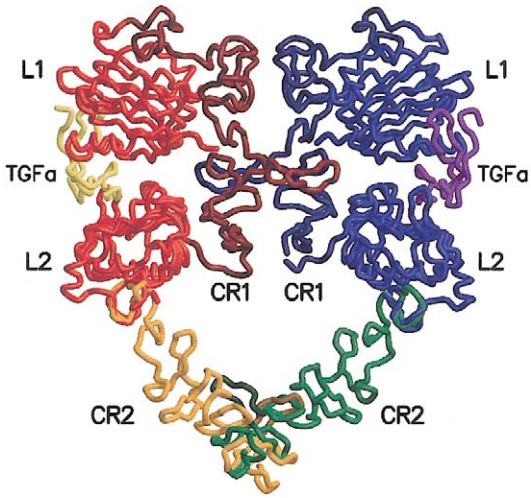

Fig. 3. Transforming growth factor-α (TGFα)-bound EGFR501 dimer with superimposed CR2 domains of ErbB3 [18–20]. Each of the proteins and protein domains are rendered as C_α worms. The EGFR501 molecules are colored red and blue with their CR2 domains colored with darker tones; the bound TGFα molecules are colored yellow and dark purple. The CR2 domains of ErbB3 were superimposed onto the epidermal growth factor (EGF) receptor fragments by using the C_α atoms of the first module of the CR2 domains of each. The two ErbB3 CR2 domains are colored orange and green except for residues 572–582, which are colored in darker tones.

of ligand binding may be to appropriately orient the L1, CR1, and L2 domains, which, in turn, position the CR2 domains and the intracellular domains of the EGFR. In this scenario, the tyrosine kinase domains are correctly positioned to enable their activation. Removal of all of the ectodomain, or residues 6–273 of the ectodomain (e.g., the Δ2–7 mutant [88–90]), results in a constitutively active complex [37,91–93]. Indeed, the Δ2–7 mutant of the EGFR has been reported to be constitutively dimerized and to have tyrosine kinase activity similar to the ligand-bound wild-type receptor [93]. While this indicates that the ectodomain, and in particular its first two domains, plays an active role in preventing kinase activation, it is unclear as to the mechanism of this inhibition.

The structure of the ErbB3 ectodomain [20] offers a view of the unliganded EGFR in its monomeric form. In the ErbB3 structure, there is an intriguing twist that allows Tyr 246 in the CR1 domain to make hydrogen bonds to the sidechains of Asp 562 and Lys 583 in the CR2 domain and hydrophobic interactions with Pro 571, Val 574, and Ile 581 also in the CR2 domain. (The equivalent residues in the EGFR are Tyr 246, Asp 563, Lys 585, Val 575, and Leu 582, respectively.) This conformation would prevent the ligand binding to both the L1 and L2 domains simultaneously and the presence of the CR1–CR2 interaction would prevent formation of the back-to-back dimer. The ErbB3 conformation is likely to place the second module of the CR1 domain (Cys 191–Cys 207 of the EGFR) close to the cell membrane and so its orientation would be completely different to that observed in the ligand-bound EGFR structure.

The minimum requirement for dimerization has been shown to be membrane-bound kinase domain itself [76]. In the absence of the ectodomain, the transmembrane-kinase form of the receptor is constitutively active. The binding of ligand to the ectodomain releases the extracellular restraints on the formation of an active kinase dimer configuration. The EGFR does not require the tyrosine kinase domain to be catalytically competent in order to dimerize [76]. The monomeric EGFR has much reduced kinase activity compared to the dimerized receptor [59,61,94,95]; it is assumed that in the absence of dimerization, the kinase is in an inactive conformation. Interestingly, deletion studies have identified the tyrosine kinase domain residues 835–918 as being necessary for formation of the dimer in the absence of ligand [87].

While many tyrosine kinases require phosphorylation of the activation loop for full enzymatic activity [96], the EGFR does not appear to be regulated at this level. Mutation of Tyr 845, the only tyrosine residue in the EGFR's activation loop, to phenylalanine does not alter the protein's kinase or autophosphorylation activities [97]. In the crystal structure, the conformation of the activation loop of the EGFR kinase in its apo-state (and also with an ATP-competitive inhibitor bound) exhibits some similarity to the phosphorylated Lck and insulin receptor kinases in the conformations of the activation loops, catalytic residues, and relative orientation of the two lobes [21,96,98]. The apo- and inhibitor-bound EGFR kinase crystal structures also show that the orientations of the two subdomains of the kinase resemble those of crystal structures of the two active tyrosine kinases. The binding of 4-anilinoquinazolines to the EGFR can induce receptor dimerization independent of ligand [99–101]. Therefore, the crystal structure conformation of the EGFR kinase complexed with the inhibitor should resemble the conformation of the kinase in the ligand-bound EGFR dimer.

Analysis of the sets of crystal contacts in the EGFR kinase structures [21] shows that the crystallographic interface with the most protein-protein contacts is between a region in the N-terminal subdomain of one copy of the EGFR kinase, including the C-helix, and a C-terminal subdomain region of a second kinase molecule, which includes its H-helix (results not shown). Interestingly, this arrangement of EGFR kinase molecules is similar to a previously proposed model of the EGFR kinase dimer [65]. This model may explain the reduction in the kinase activity of the EGFR mutant Tyr 740 → Phe; Tyr 740 is a solvent-exposed residue in the C-helix of the kinase [65,66]. The interface contains a cluster of hydrophobic residues that are largely conserved across the EGFR family and also the conserved residue Gln 911 whose sidechain makes two hydrogen bonds to the mainchain of the other kinase molecule. Although the structures of the EGFR kinase do not suggest how the EGFR is inactivated, movement of the C-helix is thought to feature in the regulation of activity of a number of protein kinases [102]. Measurements of the kinetics of stimulated and unstimulated EGFR showed that ligand binding doubles the V_{max} parameter and decreases the K_m parameter for ATP by 10-fold [103]. We propose a variation on the mechanism of activation suggested by Ge et al [103], i.e., ligand binding increases the proportion of dimerized EGFR and the reorientation of the kinase domains in a way that increases the affinity for ATP binding, probably due to conformational change, thereby enhancing the kinase activity.

Molecular targets perturbed by the activation of the EGFR

The EGFR exerts its function in the cellular environment mainly, if not exclusively, via its tyrosine kinase activity. Tyrosine phosphorylation of cellular substrates is thus the first and crucial step in transducing EGFR-mediated signals. It is often difficult to determine whether a protein, phosphorylated in response to cellular stimulation with EGF, is a direct substrate of the EGFR kinase or it is phosphorylated following EGFR-dependent activation of other cellular kinases. Given the propensity of EGFR to heterodimerize with, and activate, other members of the EGFR family [104], even direct phosphorylation in in vitro kinase assays

Table 2
Signalling proteins that associate directly with the EGFR, their function, and preferred docking sites on the EGFR[a]

Protein	Function	Docking sites on EGFR	Reference
GRB-2	Adaptor	pY^{1068}, pY^{1086}	[284]
Nck	Adaptor	ND	[285]
Crk	Adaptor	ND	[286]
Shc	Adaptor	pY^{1148}, pY^{1173}	[123]
Dok-R	Adaptor	pY^{1086}, pY^{1148}	[287]
PLC-γ	Phospholipase	pY^{1173} (N-SH2) pY^{992} (C-SH2)	[173]
P120RasGAP	Ras attenuator	ND	[288]
PTB-1B	Phosphatase	pY^{992}, pY^{1148}	[289]
SHP-1	Phosphatase	pY^{1173}	[290]
Src	Tyrosine kinase	pY^{891}, pY^{920}	[119]
Abl	Tyrosine kinase	pY^{1086}	[291]

[a] EGFR, epidermal growth factor receptor; ND, not determined.

can be confounded by the presence of heterodimers, making it difficult to unequivocally assign substrates of the EGFR kinase. For many of the proteins identified as belonging to EGF-initiated signal transduction pathways, the question of direct or indirect phosphorylation is still unresolved. One of the few phosphoproteins that are undoubtedly direct substrates of the EGFR kinase is the EGFR itself, although in a cellular context, EGFR phosphorylation and signalling can also occur through ErbB dimerization partners (see, for example, Deb et al. [105] and Ewald et al. [106]), or by activation of intracellular tyrosine kinases such as Src and JAK-2. The EGFR is autophosphorylated on five C-terminal tyrosines, most likely in an intermolecular reaction through a dimerization partner. The putative role of autophosphorylation in the maintenance of the activated state is described elsewhere; here, we will address the role of EGFR phosphorylation sites in the formation of signalling complexes, and the phosphorylation-dependent activation of major intracellular signalling pathways.

Physical association between EGFR and signalling proteins

Phosphorylation of the EGFR's C-terminus, be it autophosphorylation or transphosphorylation by other kinases such as Src and Jak-2 [97,107], provides specific docking sites for the SH2 or PTB domains of intracellular signal transducers and adaptors, leading to their colocalization and to the assembly of multicomponent signalling "particles." Signalling proteins that associate directly with the EGFR in this manner, and the EGFR tyrosines that mediate the association, are listed in Table 2. The association of other proteins with the phosphorylated EGFR is thought to be indirect (e.g., Cbl [108], PI3K-C2b [109], and Stat5b [110]), while the mode of EGFR binding for proteins such as Eps-8 and Eps-15 is still unclear. A third mode of recruitment to the EGFR occurs via the C-terminal phosphorylation sites of heterodimer partners; the sequence divergence between EGFR family members at the C-terminus allows different proteins to preferentially associate with specific EGFR heterodimer complexes, greatly enhancing the multiplicity of signals that can emanate from the set of EGFR homo- and heterodimers. This is exemplified by the p85 subunit of PI3-K, which preferentially associates with the YXXM motifs in the ErbB3 C-terminus rather than with the EGFR itself [111].

SH2 or PTB domain-mediated association of intracellular proteins with the EGFR, whether direct or indirect, is inducible and determined by the phosphorylation state of key tyrosine residues on the receptor. However, there are some proteins that are associated with the EGFR in its resting state, only to be activated or translocated to other cellular locations when ligand binds. This is the case for the zinc-binding protein ZPR-1 [112] and STAT transcription factors [113,114].

The physical association of EGFR and signalling or adaptor proteins greatly increases the efficiency of substrate phosphorylation, as well as aiding in the assembly of spatially organized multicomponent signalling complexes. It must be emphasized, however, that autophosphorylation of the EGFR is not a prerequisite for EGFR signalling; apparently normal signalling is stimulated by C-terminally truncated EGFRs expressed alone [115] or in combination with other EGFR family members [116–118]. Interestingly, an intact EGFR C-terminus has been reported to be critical for signalling initiated by amphiregulin but not EGF [118]. The ability to dispense with EGFR C-terminal phosphorylation sites is still puzzling, in view of the abundance and specificity of receptor-protein interactions mediated by the C-terminus. One could speculate that the assembly of signalling complexes can still occur in the absence of the EGFR C-terminal scaffold by association with other molecules (which in turn provide the docking sites) or by using alternative modules for binding to signalling proteins. The binding of the p85 subunit of PI3-K to the EGFR provides an example of both these modes of action. Normally EGF-dependent association of p85 with the heterodimeric complex EGFR/ErbB3 occurs via ErbB3. In this way p85 is brought in close contact with the kinase domain of the EGFR and is phosphorylated. However, direct association between p85 and the EGFR can also occur via the EGFR pY^{920}, which is located within the receptor's kinase domain and is phosphorylated by the cytosolic kinase Src [119], thus bypassing the requirement for either ErbB3 or EGFR C-terminal association sites. Similarly, phosphorylation of the transcription factor STAT5b appears to be dependent on phosphorylation of the EGFR by Src on Y^{845} [110].

Signalling pathways activated by the EGFR

Given the functional diversity of proteins that complex with, or are phosphorylated by, the EGFR, it is hardly

surprising that EGF stimulation of a cell results in the simultaneous activation of multiple pathways. These pathways are often functionally interlinked and ideally should not be considered in isolation; however, for the sake of simplicity we will discuss them individually and in particular attempt to describe the earliest steps of their EGFR-mediated activation.

Shc, Grb2, and the Ras/MAPK pathway

The cascade of biochemical events that leads from the EGFR to the activation of the proto-oncogene Ras and, eventually, of the serine/threonine kinase MAPK has been analyzed extensively. The key player in EGF-dependent Ras activation is the adaptor protein Grb2 [120]. Grb2 is constitutively bound to the Ras exchange factor Sos and is normally localized to the cytosol. Following activation of the EGFR kinase and autophosphorylation, the SH2 domain of Grb2 can bind to the EGFR. It must be noted that Grb2 can associate with the receptor either directly (via Y^{1068} and Y^{1086} [121]) or indirectly, by binding to EGFR-associated, tyrosine phosphorylated Shc [122]. It has been suggested that association of Shc to EGFR via its PTB domain, leading to its tyrosine phosphorylation and to the recruitment of Grb2, is the main step in EGF-dependent induction of the Ras/MAPK pathway [123]. However, in a different cellular system, Hashimoto et al. [124] have shown that Shc is not necessary for Ras activation by the EGFR; it is therefore still unclear whether the two modes of recruitment of Grb2 to the receptor have different functional roles or whether the predominance of one over the other is cell-type specific. In either case, relocation of the Grb2/Sos complex to the receptor at the plasma membrane facilitates the interaction of membrane-associated Ras with Sos, resulting in the exchange of Ras-bound GDP for GTP and hence in Ras activation. Activated Ras in turn activates the serine/threonine kinase Raf-1 [125]. Raf-1 activation, through a series of intermediate kinases, leads to the phosphorylation, activation, and nuclear translocation of Erk-1 and Erk-2, which catalyze the phosphorylation of nuclear transcription factors [126]. Activation of the MAP kinases also provides a negative feedback loop for this pathway since the GrB2-Sos complex is dissociated following MAPK phosphorylation of Sos [127]. This very simple outline of signalling downstream of Ras hides an incredible complexity of cross-talk between signalling pathways, feedback loops, protein relocalization, and signalling complex formation, which are beyond the scope of this article, but that have been addressed in recent reviews [128–130].

Both Grb2 and Shc play important roles in the activation of other EGFR-dependent pathways. This is due to their "modular" construction. Grb2 contains two SH2 domains and one SH3 domain, which enables it to interact with tyrosine-phosphorylated motifs as well as with proline-rich regions of other proteins (see, for example, Meisner and Czech [131]). Shc can associate with specific tyrosine-phosphorylated sequences via its SH2 and PTB domain, and, being itself phosphorylated on tyrosine by activated receptors and cytosolic tyrosine kinases, serves in turn as a binding partner for SH2-containing proteins. SH2 and SH3 domains recognize specific sequences preferentially, but not exclusively; thus, they can bind to many proteins with different affinity. For example, Grb2 has been shown to complex with proteins involved in cytoskeletal reorganization, such as FAK and dynamin [132] with negative regulators of growth factor action such as Cb1 [108], Dab-2 [133], and SOCS-1 [134], and with the inositol phosphatase SHIP [135]. Shc has also has been detected in complexes with many other proteins [136], including MEKK-1, which links it to JNK pathway activation [137], and cadherin, implying a role for Shc in cell-cell adhesion [138].

The existence of interactions between Shc and Grb2 with the EGFR, with each other, and with a subset of cellular proteins raises the questions of how interactions are controlled: Do all possible interactions occur in a single cell and, if so, does the activation of one pathway influence the activation of alternative pathways?

SH2- and SH3-mediated protein interactions are dependent on both the affinity and the relative concentration of the binding partner; high affinity interactions will be favoured at low concentrations of the target molecule, but could be displaced by low affinity interactions driven by high concentrations of alternative partners. There are many ways in which protein association patterns can vary between cell types, or within the same cell depending on the stimulus and the timing of the stimulation. Analyses of EGFR-associated signalling pathways often utilize different cell lines, and the cell type-specific levels of expression of binding partners for Grb2 or Shc may bias the detectable associations. Furthermore, when analyzing transformed cell lines it must be expected that the complexes will be different from those detected in resting cells or in cells being stimulated (e.g., during wound healing or antigenic responses). These caveats also apply to overexpression experiments, which must be interpreted with caution. It is also important to recognize that the "local" abundance of a protein may determine its availability for binding; colocalization of binding partners (e.g., at the plasma membrane) will favour interactions even when the affinity is low. It is well established that many signalling proteins relocalize within the cell following stimulation with growth factors; presumably this relocalization plays a significant role in controlling the timing and compartmentalization of protein-protein interactions. Finally, posttranslational modifications of proteins may alter the affinity of specific interactions (as is the case for Sos and Grb2), and allow alternative complexes to form.

Recently, interactions between proteins have been studied directly in cells using GFP/YFP fusion proteins and FRET analysis [139]. Provided the levels of the transfected proteins remains in the physiological range, this technique offers considerable promise for studying the formation and

localization of protein complexes following stimulation of a cell with ligands such as EGF.

The Src family of kinases

c-Src and other members of this family of cytosolic tyrosine kinases have long been implicated in signal transduction from polypeptide growth factor receptors such as the EGFR (reviewed by Belsches et al. [140]). In the case of EGFR signalling, the evidence for an involvement of members of the Src family of kinases is overwhelming. Overexpression of Src proteins strongly enhances EGF-mediated proliferation and transformation in fibroblasts and epithelial cells [141,142]. Conversely, inhibition of Src activity by microinjection of antibodies, by dominant negative Src kinase constructs or by exposure of the cells to Src-specific pharmacological inhibitors, can block EGF-dependent DNA synthesis [143,144] and reverses the transformed phenotype of EGFR- or ErbB2-overexpressing cells [145]. However, it is still not clear whether Src is a signal transducer downstream of the EGFR or a contributor to EGFR activation. There is evidence to support both models.

In A431 cells and in colon carcinoma cell lines, endogenous c-Src has constitutively elevated kinase activity, which is reduced to basal levels by the EGFR-specific kinase inhibitor AG1478 [146,147]. EGF-dependent Src kinase activation is observed in cells that overexpress the EGFR, such as A431; in these cells it is also possible to detect the association of Src and the receptor, while in cells that do not overexpress either EGFR or Src, association between the two proteins has been difficult to prove. The association is most likely direct, and mediated via the Src SH2 domain, although the exact binding site on the EGFR is still unclear. Using in vitro assays with the two purified kinases. Stover et al. [119] have shown that Src does not bind to the major autophosphorylation sites of the EGFR, but phosphorylates novel sites within the kinase domain of the receptor (Y^{891}) and (Y^{920}). These two phosphotyrosines bind the SH2 domain of Src, and Y^{920} may also provide a docking site for the p85 subunit of PI3-K. In these experiments, Src-phosphorylated EGFR, but not autophosphorylated EGFR, caused a marked activation of Csk-inactivated Src kinase activity. Two other Src-dependent phosphorylation sites have recently been identified within the EGFR, i.e., Y^{845} and Y^{1101} [148]. pY^{1101} is a potential Src-binding site [149], while pY^{845} appears necessary for STAT5b activation [110]. The physiological relevance of Src providing its own EGFR phosphorylation sites for docking has been put in doubt by recent results, in which binding of Src to the EGFR was found to be independent of Src kinase activity [97].

The possibility that phosphorylation by Src may contribute significantly to the activation of EGFR is of great interest. Src phosphorylates the EGFR on Y^{845} both in vitro and in vivo [148,150]; this residue, located on the activation loop of the EGFR kinase domain, is highly conserved in tyrosine kinases, and plays a crucial role in the activation of receptor kinases such as KDR [151]. Trk [152], and insulin receptor [34]. The role of this phosphorylation site in EGF-mitogenic signalling is controversial; Gotoh et al. [153] found no effect of $Y^{845}F$ mutation on EGF-mediated proliferation and transformation in fibroblasts, while Tice et al. [97] found that the same mutations abolishes not only EGF-dependent, but also serum-dependent, stimulation of DNA synthesis. The ability of Y^{845F}-EGFR to autophosphorylate or phosphorylate She did not appear to be affected. suggesting that the mutant receptor maintains tyrosine kinase activity. However, both C-terminal phosphorylation of the EGFR and She phosphorylation have been shown to occur in cells expressing kinase-negative EGFR, without concomitant stimulation of DNA synthesis [154]. In this case, the phosphorylation appears to be caused by heterodimerization between the mutant EGFR and ErbB2. It is therefore difficult to address the question of Src-mediated EGFR kinase activation in any cell that coexpresses EGFR and one of its heterodimer partners, ErbB2 or ErbB4.

The Src and EGFR tyrosine kinases share many substrates, again making it difficult to discriminate between Src-mediated and EGFR-mediated signalling following stimulation with EGF (reviewed by Belsches et al. [140]). EGFR and the She protein are phosphorylated by both kinases, but the phosphorylation occurs at different sites [155], potentially enhancing the spectrum of She-mediated responses from the EGFR. p120RasGAP is also a substrate for both kinases, while p190RhoGAP has been shown to be selectively phosphorylated by c-Src [156]. Since the association between p120RasGAP and p190RhoGAP is implicated in EGF-mediated cytoskeletal rearrangement, and is mostly dependent on phosphorylation of RhoGAP by Src [157], c-Src appears to act as a downstream signal transducer from EGFR. There is indeed circumstantial evidence that most of the cytoskeletal reorganization that follows stimulation of cells by EGF is mediated by preferential substrates of the c-Src kinase; these include FAK [132], p130Cas [158], cortactin [159], EAST [160], and Eps-8 [161].

Activated c-Src is also intimately linked to the activation of PI3-K. As mentioned above, Src-dependent phosphorylation of the EGFR molecule can provide a docking site for p85, presumably facilitating its phosphorylation by EGFR and the consequent activation of PI3-K. Src also directly phosphorylates and activates PI3-K [162], once again pointing to the large overlap in signal activation by Src and EGFR and to the difficulties of unequivocally assigning specific pathway activation to either kinase. The development of truly specific tyrosine kinase inhibitors will be of great help in dissecting the relative roles of c-Src and EGFR kinases in a cellular context.

The JAKs and STATs pathways

STATs were first identified as signal transducers downstream of cytokine receptors (reviewed by Darnell [163] and

Ihle et al. [164]). In mammals, seven STAT genes have been identified (STAT 1 to 4, 5a, 5b, and STAT6). STAT proteins are inactive transcription factors, which are activated and translocated to the nucleus upon specific receptor stimulation. Classically, STATs are recruited to the intracellular domain of the cytokine receptors through specific binding between STAT SH2 domains and receptor phosphotyrosine residues. Homo- and heterodimerization of STAT proteins is a prerequisite for activation and translocation to the nucleus, and is mediated by tyrosine phosphorylation of critical residues (Y^{699} in STAT5b, Y^{694} in STAT5a, and Y^{701} in STAT1); further residues have also been implicated in the activation of STAT5b (see Kloth et al. [110]). In cytokine signalling, activation is mediated by the JAK family of kinases (reviewed by Leonard [165]). STAT proteins, in particular STAT-1, 3, and 5, have also been implicated in EGFR signalling; however, the mode of activation appears to be significantly different from that used by cytokine receptors. First, the ligand-dependent phosphorylation of STATs by EGFR does not require JAK kinases [166–168]. Second, STATs do not bind to the C-terminal phosphotyrosines of the EGFR; indeed it appears that STATs are constitutively associated with the EGFR [113,114]. However, as in JAK kinase signalling, activation of STAT transcriptional activity is strictly dependent upon the EGFR tyrosine kinase activity [167]. More recent reports have implicated the Src kinase in EGF-dependent STAT activation [110,114], but it is unclear whether Src acts upstream or downstream of EGFR activation in this case.

Phospholipid metabolism: PLD, PLCγ, and PI3-K

EGF stimulation of a cell has marked effects on its phospholipid metabolism, including phosphatidylinositol turnover and production of phosphatidic acid (PA) and arachidonic acid (AA). Of the enzymes involved in these pathways, at least three can be activated directly by the EGFR, i.e., phospholipase C-γ (PLCγ), phosphatidylinositol-3-kinase (PI3-K), and phospholipase D (PLD), while others, such as phospholipase A2, are regulated indirectly by EGF-mediated activation of other pathways.

PLD hydrolyses phosphatidylcholine to generate choline and the second messenger PA (reviewed by Houle and Bourgoin [169]). PLD activity is stimulated in whole cells by EGF treatment, but until recently the stimulation was thought to be indirect and mediated by cofactors such as PKC, Rho, and phosphotidylinositol biphosphate (PIP_2). While this may indeed be the case for PLD_1, PLD_2 has now been shown to be associated with, and activated by, the EGFR [170]. The mechanism of activation, while still obscure, appears to require the physical association of PLD with EGFR but not necessarily tyrosine phosphorylation; although Y^{11} of PLD_2 has been identified as the major site of phosphorylation by the EGFR kinase, mutations at this residue do not abolish activation [170]. Activation of PLD may require a conformational change that is stabilized, but not induced, by tyrosine phosphorylation; a similar mode of activation (dependent on complex formation but independent of tyrosine phosphorylation) has been proposed for PLC γ[171], suggesting a common mechanism of activation for this class of molecules.

PLCγ (reviewed by Kamat and Carpenter [172]) binds directly to the autophosphorylated EGFR via Y^{1173} and Y^{992} [173] and is phosphorylated by the EGFR kinase on Y^{771} and Y^{1254} [174]. The exact mode of PLCγ activation by the EGFR is not clear; reportedly it requires direct association with the receptor but not necessarily tyrosine phosphorylation [171]. Once activated, PLCγ catalyzes the hydrolysis of PtdIns(4,5)-P_2 to yield the important second messengers 1,2-diacylglycerol (DAG) and inositol 1,3,5-trisphosphate (IP_3). IP_3 mediates calcium release from intracellular stores, affecting a host of Ca^{2+}-dependent enzymes, while DAG is a cofactor for the activation of the serine/threonine kinase PKC. Thus, through IP_3, EGFR can activate Ca^{2+}-dependent pathways such as RaI [175] and NFκB ([176]), and through PKC multiple signalling components, including the MAPK and JNK pathways [177,178] and possibly the Na^+/H^+ exchanger [179].

Phosphoinositide-3-kinases are major players in cellular functions, where they contribute to a variety of cellular processes including proliferation, survival, adhesion, and migration (reviewed by Cantley [180]). PI3-kinases catalyse phosphorylation on the 3′ position of phosphatidylinositols (PtdIns) and are assigned to three classes according to their subunit structure and their preferred lipid substrate (reviewed by Djordjevic and Driscoll [181]). Of the three classes of typical PI3-kinases, only class Ia is activated by tyrosine kinase receptors. Interaction between PI3-kinase and the ErbB receptors is required for activation, and is mediated by association of the phosphorylated receptors with the p85 subunit of PI3-K via the latter's SH2 domain [182]. As mentioned previously, the major binding partner of p85 is not the EGFR, but ErbB3 [183,184]; however, activation of PI3-K is observed in response to EGFR ligands through formation of ErbB1/ErbB3 heterodimers, as well as potentially by Src phosphorylation of the EGFR, so it is relevant to this discussion of EGF-mediated signalling pathways.

PI3-K Ia generates phosphatidylinositol-3,4,5-trisphosphate (PIP_3). One of the best characterized targets of this second messenger is the Ser/Thr kinase Akt (PKB [185]), which binds to the lipid and is translocated to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 (PDK-1) and possibly other kinases (reviewed by Nicholson and Anderson [186]). PKB/Akt is a major mediator of PI3-K action in survival and proliferation, and may well be the major mediator of the antiapoptotic effects of EGFR activation.

Recently, the crystal structure of the catalytic subunit (p110) of PI3-Kγ in complex with Ras has been solved [187]. The structure shows a change in conformation of the catalytic p110 upon binding to Ras, consistent with a Ras-

mediated activation model. Since activated Ras is one of the major downstream effectors of EGFR signalling, this mechanism may represent yet another way in which activated EGFR regulates PI3-kinase activity.

The role of the EGF family of ligands and EGFR in mammalian physiology and pathology

A vast body of knowledge has been accumulating in recent years on the role of the EGF family of ligands and receptors in embryonic development, physiology, and pathology. Thanks to the power of genetic screens, much of the progress on the developmental role of the EGF/EGFR system has come from studies on invertebrates, such as *Drosophila* and *C. elegans.* The developmental aspects of EGF/EGFR signalling, both in invertebrates and in mammals, are covered elsewhere in this issue (Shilo and Sternberg). In this article we will concentrate on the role of EGF ligands and receptors in newborn and adult mammals.

Murine EGF and its human equivalent, β-urogastrone, were first isolated and identified because of their effects on tooth eruption and eyelid opening [188] or inhibition of gastric acid secretion [189], respectively. Another family member, TGFα, was identified as a component of "sarcoma growth factor," produced by retrovirally transformed fibroblasts [190]. Attempts to determine the physiological role of EGF and TGFα in in vivo studies date back to the early 1980s. Initially, the ligands were injected in neonatal mice, and physiological changes were monitored. The most striking effects of EGF were precocious eyelid opening and tooth eruption [191,192], although more subtle effects on neurobehavioural development [193] and, unexpectedly, a reduction in growth rates [193,194] were also observed. With the analysis of natural mouse mutants, the development of transgene technology, and the advent of gene targeting in murine ES cells, the study of gain-of-function or loss-of-function in the EGF/EGFR axis became much easier and led to a clearer understanding of the role of these ligands and receptors in mammalian physiology and pathology. It must be emphasized, however, that the effects of altering the EGF/EGFR axis may be indirect; for example, EGF and TGFα modulate hormonal responses such as the release of luteinizing hormone and thyroid hormone, and the EGFR is required in mediating many of the effects of estrogen.

Gain-of-function: EGFR and its ligands

Apart from the in vitro data, suggesting a role of EGF/EGFR in cell proliferation, evidence has been accumulating that overexpression of the ligands and/or receptors, as well as ligand-independent receptor activation, occurs in many epithelial cancers, most notably gliomas and breast, pancreas, and liver carcinoma. What is not clear is whether this overexpression/activation is indeed causative for the formation of tumours or occurs during tumour progression. The use of transgenic animals has allowed the role of these proteins to be addressed. Of the EGF family ligands, TGFα has been the most studied by using this technology.

Targeting of TGFα to the skin by means of keratin promoters results in hypertrophy and hyperkeratosis accompanied by alopecia or stunted hair growth. The scaly skin and localized leukocyte infiltration are reminiscent of psoriasis [195,196]. The psoriasis-like lesions and hyperkeratosis are even more prominent in mice expressing a K14-amphiregulin transgene [197], strengthening the case for involvement of EGFR activation in this skin condition. Interestingly, TGFα transgene expression is linked to the appearance of papillomas following irritation or wounding, but without progression to carcinomas [195,196]. Inducible expression of the TGFα transgene in the kidney, as a model for polycystic kidney disease (PKD), has been linked to the formation of renal cysts and accelerated progression of the disease in a strain of mice predisposed to PKD, but not to the onset of polycystic kidneys [198,199]. Targeted overexpression of TGFα in the mammary gland results in hyperplasia, cystic expansion, and papillary adenomas following multiple pregnancies and lactation. Expression of TGFα in these mice inhibits involution of the mammary gland after pregnancy and lactation, resulting in hyperplastic alveoli in multiparous females; however, the incidence of tumour formation, while variable, is generally low (see for example, Davies et al. [200] and Sandgren et al. [201]). It appears therefore that overexpression of TGFα is linked to hyperproliferative responses but does not generally lead to tumours in rodents. Even in transgene models where TGFα-associated tumours are observed, the latency period tends to be long and the incidence low, suggesting that the TGFα/EGFR system provides only one of the steps in multistage carcinogenesis, and neoplastic transformation only occurs when other genes within the target tissue are also mutated.

Apart from the ras oncogene and the components of the EGF/EGFR family of ligands and receptors, the most commonly amplified oncogene in breast cancer is c-myc [202–204]. Studies using double transgenic mice have addressed the significance of EGF system/c-myc interactions in the mammary gland [201,205]. Transgenic mice that overexpress both TGFα and c-myc develop mammary tumors irrespective of the sex, while in transgenic mice expressing c-myc alone, tumor formation only occurs in females, and even then with much reduced frequency and with a long latency periods. The cooperativity between c-myc and TGFα has been attributed to an antiapoptotic effect exerted by TGFα, coupled with increased proliferative responses associated with c-myc overexpression [205]. A strong antiapoptotic effect of TGFα could also help explain the lack of involution of the mammary gland described in mice overexpressing TGFα [200].

Even more striking is the cooperativity between TGFα and c-myc in the induction of hepatocarcinomas. TGFα

transgenic mice develop hepatic tumors at low frequency and with very long latency. Overexpression of c-myc in these mice dramatically shortens the latency period and accelerates tumor growth [201,206,207]. The hepatocellular carcinomas from c-myc/TGFα transgenic mice display a very low apoptotic index compared to hepatocarcinomas from TGFα or c-myc single transgenic mice, present abnormalities in cell-cycle protein expression [208], and overt tumor formation is often preceded by aneuploidy and chromosomal breakages [209].

The commonly observed features in these models of TGFα overexpression are an enhancement of cellular proliferation, as evidenced by increased mitotic index, and a reduction in the rate of apoptosis, resulting in hyperplasia of skin, mammary glands, and liver. Thus, TGFα appears to be capable of altering the balance between cellular proliferation and death. Stimulation of the EGFR pathways may override the DNA damage check points, resulting in decreased apoptosis and in the accumulation of secondary mutations.

Confirmation of this link between overactivation of the EGFR and transformation should come from experiments where the EGFR is either overexpressed or constitutively activated. There is ample documentary evidence of EGFR overexpression or activation in spontaneous tumours, as well as numerous studies on the tumorigenicity of cells lines expressing activated EGFR in mouse models. Of particular interest in this context is a naturally occurring deletion mutant of the EGFR, Δ2–7 (also called EGFRvIII). EGFRvIII is the most common EGFR mutation in human cancers, having been detected in 40–50% of grade VI glioblastomas [210] and in up to 70% of medulloblastomas and a small proportion of breast and ovarian carcinomas [211]. EGFRvIII arises from a genomic deletion of exons 2–7 [212,213], resulting in a protein that lacks most of the extracellular domain. As a result, EGFRvIII is not activated by ligand; however, it is constitutively activated and is not internalized, which results in constitutive long-term signalling [37]. Transfection of glioma cell lines with EGFRvIII dramatically increases their tumorigenicity in nude mice [214]. The tumorigenic potential of cells expressing EGFRvIII has been linked to upregulation of Bcl-X_L and resulting inhibition of apoptosis [215]. However, constitutive activation of the EGFR may not be sufficient to initiate and maintain the transformed phenotype. The experiments described above were performed by transfecting the activated receptor in cells adapted to continuous growth in culture, which are likely to harbour complementing mutations. When the expression of an activated EGFR is directly targeted to glial progenitor cells in mouse models, there is no evidence of increased rates of tumour formation. However, expression of the activated receptor in mice genetically defective in cell-cycle inhibitor proteins (such as INK4a) does lead to the development of gliomas [216]. These findings have been confirmed and extended by Bachoo et al. [217]; EGFR activation in INK4a/Arf-deficient mice leads to dedifferentiation of astrocytes and is instrumental in gliomagenesis.

Effects of the loss of EGFR function

While the gain-of-function experiments address mainly the role of the EGF/EGFR system in abnormal proliferation, loss-of-function mice have shed some light on the developmental and physiological role of the system. Since EGF is produced by the submaxillary glands, sialoadenectomy was initially used as a tool to investigate the effects of reduced EGF levels in vivo. In these studies the organs most affected were the mammary gland [218] and the epidermis [219]. In both organs there was a reduction in size and thickness, which could be reversed by administration of EGF. In these early experiments, however, the levels of EGF were reduced but not abolished completely. Genetically null animals have been created by targeted inactivation of the EGF, amphiregulin, and TGFα genes. Surprisingly, in view of the pleiotropic role of TGFα, the phenotype of the TGFα-null animals is very mild. The most striking abnormalities are found in the skin architecture and in the development of the eyes; the hair follicles are deformed resulting in wavy fur and whiskers, and the eyes of the TGFα-null mice are open at birth and are opaque [220,221]. More recently, a significant reduction in the number of dopaminergic neurons in the substantia nigra of TGFα knockout mice has also been described [222].

Recently, Luetteke and colleagues [223] have published the results of targeted inactivation of the genes coding for other ligand in the EGF family. Their analysis covers single, double, and triple deletions of the EGF, amphiregulin (AR), and TGFα genes. EGF-null or AR-null mice display no obvious phenotype, not even the wavy coat characteristic of the TGFα-null mice. The lack of phenotype contrasts with the results obtained by reducing EGF levels via sialoadenectomy, and may be in part due to compensatory mechanisms, such as upregulation of other EGF family ligands, in animals chronically deficient for the growth factor and/or nutritional problems with the sialoadonectomized mice. Double- and triple-null mice were generated by intercrosses. Compared to the original TGFα knockouts, triple-null mice have an increased penetrance of eye defects, dermatitis, and skin ulcerations with aging. However, only the absence of AR, combined with absence of either EGF or TGFα, results in impaired mammary gland development. Interestingly, the defects in duct formation and lobuloalveolar development were not attributable to decreased proliferation or increased apoptosis, but it has been suggested that the defects were probably the result of abnormal epithelial cell migration. In these experiments, three of the six known EGFR ligands have been ablated, and yet the phenotype is relatively mild. Again, it is possible that upregulation of the other EGF family members (e.g., HB-EGF or betacellulin) could still

regulate the physiological activation of the EGFR in most tissues.

Inactivation of the EGFR, the common signalling partner for EGF, TGFα, HB-EGF, AR, betacellulin, and epiregulin, should prevent compensatory mechanisms involving ligand overexpression from masking the effects of ligand deficiency. Indeed, EGFR knockout mice are severely affected, although to varying degrees depending on their genetic background [224–226]. At its most severe, the lack of EGFR causes peri-implantation or mid-gestational death. In some strains, however, the mice survive up to 3 weeks post birth; these animals show severe abnormalities of skin, lungs, the gastrointestinal tract, brain, and liver [224], confirming the importance of the EGF/EGFR system in epithelial cell regulation. EGFR-null mice show disorganized hair follicles and curly coats. This phenotype also occurs in mice expressing a dominant-negative EGFR construct targeted to the skin, and in the naturally occurring mouse strain *wa-2*, which carries an inactivating mutation in the EGFR gene [64,227]. This waviness of fur and whiskers is characteristic of the TGFα-null mice, but is not present in EGF knockouts, implying that TGFα is a major physiological ligand regulating the activation of the EGFR in the skin.

Given the very short life span of the EGFR-null mice, a study of mammary gland development in these mice is not possible. The effects of EGFR loss-of- function in this tissue have been studied instead by targeting a dominant-negative EGFR to the mammary gland. Expression of the dominant-negative EGFR construct inhibits ductal branching and outgrowth in virgin mice, although postpartum lactation still occurs, probably following upregulation of the endogenous wild-type EGFR [228]. The mammary glands of mice expressing *wa-2* EGFR mutation are reduced in size and have underdeveloped ducts ([64]; K.J. Fowler, personal communication). Using transplants of neonatal mammary glands from EGFR-null mice into normal mice, and in tissue recombination experiments. Wiesen and coworkers [229] concluded that EGFR presence in the stroma, rather than the epithelium, is essential for ductal growth and branching. The impairment of ductal morphogenesis is not apparent in EGF or TGFα-null mice but is characteristic of the AR knockout mice; this implicates amphiregulin as a key EGFR ligand in mammary tissue.

The tissue specificity of these phenotypes suggests that individual EGFR ligands are important in particular tissues, or at particular stages of development. Alternatively, the specificity may be conferred by selective coexpression of EGFR family members in a given tissue. In the latter case, two further alternatives are possible, i.e., the same ligand, binding to different receptor combinations, triggers tissue-specific responses, or different receptor combinations preferentially bind to, and are activated by, selected EGF family ligands. More information is needed, from both in vivo and in vitro models, before these challenges can be answered and the complexities of signalling from the EGF/EGFR family of ligands and receptors in a physiological setting can be understood in detail.

Cell motility: EGF receptor–integrin cooperativity

Cell migration is a complex, coordinated process that allows cells to reach specific destinations during embryonic development, to maintain the cellular architecture of self-renewing tissues, repair wounds, and to defend against infectious agents [230–232]. Signals from several classes of receptors play critical roles in the regulation of cell movement; integrins, through their ability to signal and form adhesive contacts linking the extracellular matrix (ECM) and the actin cytoskeleton [233–235], growth factor receptors activated through either autocrine or paracrine pathways, also regulating the actin cytoskeleton [236], and chemotactic receptors [237]. The involvement of EGF receptor signaling in various normal physiological processes requiring cell movement and deregulation of the motility response in pathological conditions such as tumour invasion is well documented and has been reviewed by others [238–241]. Our understanding of the mechanisms by which signals from the EGF receptor modulate cell locomotion has progressed significantly over the past decade and will be briefly summarized below. Where appropriate we have referred to other excellent recent reviews for more in-depth discussions of particular topics.

Although EGF receptor stimulation can lead to both cell proliferation and migration [2], these responses are separable and mediated via different signalling pathways [242]. A series of reports by Wells and coworkers ([242–244] and reviewed by Wells [2]) linked a PLCγ-dependent pathway to cell motility triggered by activation of the EGF receptor. Using cells expressing various receptor mutants that differ in their ability to induce a motility or mitogenic response, they found that the motility response elicited by EGF receptor stimulation requires kinase activity of the receptor and the presence of at least one autophosphorylation site, tyrosine^{992} [243]. The ability of EGF to induce cell movement correlates with the activation of PLCγ and movement can be inhibited by blocking the function of this enzyme [242]. The observation that EGF stimulates MAP kinase activation in both motogenic and nonmotogenic EGF receptor-expressing cells is consistent with the current view that the motility and mitogenic responses elicited by the EGF receptor diverge at the immediate postreceptor level and that MAP kinase activation alone is not sufficient to induce a motility response [242]. Further work by Chen and colleagues [244] provided evidence that EGF-induced activation of PLCγ stimulates cell motility by releasing PIP_2-bound gelsolin (and possibly other actin-modifying proteins, profilin, cofilin, and CapG) from the membrane, thereby restoring its ability to bind, sever, and cap polymerized actin filaments, a process required for filopodia/lamel-

lipodia extension and retraction in motile cells [245]. Thus, EGF receptor-mediated activation of PLCγ is believed to be critical for the reorganization of the actin cytoskeleton and contribute to the initiation of the asymmetric motile phenotype (reviewed by Wells et al. [238]).

Although not sufficient to stimulate motility by itself, MAP kinase may in part regulate cell motility by modulating integrin adhesive functions. Expression of activated mutants of H-Ras or its kinase effector Raf-1 in CHO cells expressing chimeric integrins suppresses integrin activation (ligand binding affinity). The suppression of integrin activity correlates with MAP kinase activation and appears to result in loss of cell spreading [246]. The downstream elements of this pathway have yet to be identified and it is still unclear how MAP kinase activation relates specifically to EGF receptor-mediated cell motility. One possibility is that MAP kinase stimulates the disassembly of focal adhesions [247]. Of course, disassembly of the focal adhesions results in decreased cell adhesion to the substratum. The effect of EGF in fibroblasts can be inhibited by blocking MAP kinase activation with the MEK inhibitor, PD98059. Together these studies implicate the MAP kinase pathway in both weakening of adhesion associated with initial motility shape change [246] and as an effector of deadhesion of the uropod [230,248].

The family of intracellular calcium-dependent proteases, calpains, is also important for focal deadhesion necessary during retraction of the trailing edge of migrating cells. Inhibition of calpains results in an elongated but ultimately immobile cell [249,250]. Calpain activation in EGF-stimulated dermal fibroblasts coincides with cell compaction, detachment, and enhanced motility [250]. The enzyme could be critical for cell movement under conditions where detachment of the trailing edge of motile cells becomes rate limiting, e.g., in situations where there is high substrate adhesiveness [249,251]. The events leading to activation of calpain are still incompletely understood and may differ between isoforms. At least two isoforms of calpain, including μ-calpain and M-calpain, have been implicated in cell motility through their ability to cleave several proteins found in adhesion complexes (reviewed by Glading et al. [252]). The former may be critical for integrin-mediated motility (haptokinesis [249]) and appears to be more sensitive to activation by Ca^{2+} fluxes, possibly triggered through stretch-activated calcium channels as demonstrated in fish keratocytes [253]. M-calpain is the more likely isoform downstream of growth factor receptor-mediated cell motility but its activation in vitro requires Ca^{2+} concentrations that appear unlikely to be attained under physiological conditions. Hence, a number of alternative or complementary mechanisms have been proposed for activation of M-calpain by growth factor receptors (reviewed by Glading et al. [252]).

Using a combination of antisense oligonucleotides, inactive mutants, activated mutants, or specific inhibitors of MEK, ERK1, ERK2, and myosin light chain kinase (MLCK), Klemke et al. [254] linked the EGF receptor, MAP kinase activation, and myosin-mediated contraction forces. The model suggests that basal and EGF-directed haptotactic responses involve direct phosphorylation of MLCK by MAP kinase. This phosphorylation event enhances MLCK's ability to phosphorylate the myosin regulatory light chain, thereby promoting myosin ATPase activity and polymerization of actin cables, a well-established role for myosin-II in the modulation cell contraction [255]. In contrast to the study by Hughes et al. [246], the MAP kinase-MLCK pathway appears to be independent of initial adhesion and spreading of cells on coated ECMs [254]. Although the authors did not examine whether the increased motility was a result of cell:substratum detachment or cell body translocation, recent evidence showing that the kinase inhibitor H-7 or the specific MLCK inhibitor ML-7 induces dissolution of focal adhesion plaques in REF52 fibroblasts [256]. These results suggest that the primary role of the EGF-induced MAP kinase-MLCK pathway in cell locomotion would be in the translocation of the cell body and/or retraction (rather than deadhesion) of the trailing edge of migrating cells.

The role of PI3-K in EGF-mediated cell movement is less clear. Treatment of human breast cancer cells and NIH 3T3 fibroblasts overexpressing the EGF receptor with PI3-K inhibitors was reported to enhance EGF-stimulated haptokinesis. The increased rates of cell movement were postulated to be due to increased adhesiveness [257]. In contrast, wortmannin partially inhibits EGF-directed chemokinesis of bladder carcinoma cells [258]. Similarly, attenuation EGF receptor-dependent PI3-K activation by overexpression of SIRPα1 in U87MG glioblastoma cells blocks their haptotactic migration [259]. The discrepancies between these studies may reflect differences in the signalling networks of the three cell types. Alternatively, the difference may depend on the contribution of PI3-K to integrin- and growth factor-mediated motility (haptokinesis vs. chemokinesis) and the linkage of these pathways to the Rho-like GTPases. The role of Rho-like GTPases Rho, Rac, and Cdc42 in integrin-mediated actin cytoskeleton remodelling and cell movement has been studied extensively. Rho is believed to regulate stress fibers and focal adhesion formation while Rac and Cdc42 control the formation of lamellipodia and filopodia/cell polarity, respectively [260–263].

Rac and Rho appear to have antagonistic roles on cell morphology by controlling the actinomyosin cytoskeleton via phosphorylation of myosin heavy chain and regulatory light chain, respectively [245]. In A431 cells, EGF stimulates motility and this response in associated with Rho-dependent cell rounding and cortical actin polymerization and Rac-dependent membrane ruffling and lamellipodia formation [264]. Further evidence suggests that integrin influences on motility require PI3-K downstream of Rac and Cdc42 [235,265,266]. In contrast, PI3-K acts upstream of Rac in growth factor-mediated motility, presumably by activating GTPase exchange factors through 3′-phospho-

inositides [267–270]. Lipid products of PI3-K may also regulate the adhesive strength and contribute to EGF-mediated cell motility by two alternative mechanisms, both involving remodeling of the actin cytoskeleton. First, activation of PI3-K upon PDGF stimulation was found to induce restructuring of focal adhesion plaques in rat embryonic fibroblasts and this effect could be mimicked by PtdIns(3,4,5)-P3 [271]. The authors provided evidence that PtdIns(3,4,5)-P3 mediates its effect by disrupting the interaction between α-actinin and integrin β-subunit. It is thus conceivable that PI3-K lipid products may act in an analogous way in response to EGF stimulation although this remains to be demonstrated. More recently, Piccolo et al. [272] reported that EGF-mediated activation of PI3-K leads to translocation of PLCγ1 to the leading edge of migrating cells in a wound-healing assay. Migration could be inhibited by expression of the pleckstrin homology domain of PLCγ1, providing evidence that this effect was mediated by direct interaction between PLCγ PH domain and PtdIns(3,4,5)-P3 as reported by others. EGF/PI3-K pathways are likely to be critical for the establishment of cell polarity during EGFR-mediated chemotaxis [273].

Clearly, more work is required to elucidate the precise contribution of PI3-K to EGF or other growth factor effects on cell motility. It is noteworthy, however, that the 3′-phosphoinositide products of PI3-K activate the proto-oncogene Akt, which in turn phosphorylates GSK3, preventing the formation of GSK3/APC/β-catenin complexes [274, 275]. Thus, EGF receptor-activated PI3-K could regulate the function of both β-catenin and APC. The latter has been implicated in the migration of normal epithelial cells in the intestinal crypts and localizes at the tip of microtubules in actively migrating regions of epithelial cell membranes [276]. Although its precise function remains elusive, the potential role of APC in EGF-mediated cell migration is supported by the recent observation that EGF induces migration of murine colon epithelial cells in an APC genotype-dependent manner [277].

Although it is clear that signals from growth factors and integrins are intimately linked and often overlapping [233–235], the complex relationship between these classes of receptors and how they cooperate to control cell movement is still the subject of intensive investigation. Recent studies have shed some light on this issue. The chemokinetic response of fibroblasts to EGF depends on the substratum concentration, which influences both the speed and directional persistence of migration [278]. A recent study by Gu et al. [279] addressed the role of the lipid/protein tyrosine phosphatase PTEN in integrin-mediated cell migration. Using various inhibitors and activators of integrin signaling, they defined two pathways that diverge at the level of Shc and FAK in glioblastomas. Their evidence indicates that the Shc-MAP kinase pathway regulates integrin-mediated random migration, whereas the FAK-p130^{Cas} pathway regulates directional migration. Both these pathways are inhibited by PTEN, which results in reduced rate and persistence of cell migration. Interestingly, PTEN was shown to associate with Shc and to directly dephosphorylate Shc (Tyr^{239} and Tyr^{317}). These Tyr residues are phosphorylated after the EGF receptor is activated. Studies in keratinocytes using galvanotaxis assay (cathodal migration of cells in an electric field) support the involvement of the EGF receptor in the regulation of directed migration. Interfering with MAP kinase activation with PD98059 completely abolishes the effect of EGF on the rate of migration but only partially blocks directional migration on laminin or fibronectin, indicating that other signals (from integrin and/or EGF receptor) are required to control directionality [280]. Most important, this and another study [281] have shown that directional migration of keratinocytes requires kinase activity and redistribution of the EGF receptor at the leading edge, resulting in asymmetric actin polymerization in migrating cells [282]. Specific inhibition of the EGF receptor kinase activity with low concentrations of PD158780 blocks directional migration. Higher concentrations are required to block the rate of random migration, indicating that the rate of migration is controlled by multiple kinase effectors. The importance of EGF receptor redistribution in directional migration is supported by the work of Li et al. [283]. Using chemotaxis chambers, these authors observed that EGF is required to be copositioned with ECM proteins (in the bottom chamber) to stimulate the haptotactic migration of B82L fibroblasts. EGF added directly to the cells (in the upper chamber) decreased their maximal migration toward the gradient of EGF/ECM. Most important, haptotactic migration toward fibronectin requires the presence of active EGF receptors even in the absence of apparent autocrine or exogenous ligand stimulation. Copresentation of EGF and ECM components enhances the polarization events required for directional migration and facilitates cross-talk between integrin and EGF receptors at the leading edge.

In summary, multiple signalling pathways are generated by activation of the EGF receptor and these pathways control the distinct steps of the motility process. Further studies are required to clarify the precise contribution of PI3-K and APC to EGF-mediated cell motility and how the various pathways are temporally integrated. Most of the studies described above have been performed in fibroblasts or keratinocytes and data for several other cellular systems will be required for a more complete understanding of the molecular regulation of the movement of mammalian cells.

Concluding notes

Much progress has been made in understanding the mechanism of EGFR activation upon ligand binding. However, there are many basic questions that must be answered about the nature of the EGFR on the cell surface, i.e., the nature of the inactive, unliganded EGFR monomer and dimer: how ligand induces the conformational transition in the ectodomain; how ligand binding stimulates the activa-

tion of the kinase; and whether mechanisms such as secondary dimerization or the formation of higher order complexes regulate the activation on EGFR homodimers and ErbB hetero-oligomers. The three-dimensional structures for parts of the EGFR have already led to a much clearer understanding of the activation processes. Mutation experiments will soon lead to a more detailed understanding of the mechanisms regulating the action of the EGFR. It is critical that these experiments are performed under conditions where the normal regulatory mechanisms of receptor activation, turnover, and signalling apply and that the effects of heterodimerization are controlled. The EGFR is a complex signalling system important in normal physiology and in the maintenance of the tumorigenic state. Studies of its biochemistry and biology have already made deep contributions to cell signalling and there are bound to be many more surprises in the near future.

References

[1] G. Carpenter, S. Cohen, Epidermal growth factor, J. Biol. Chem. 265 (1990) 7709–7712.

[2] A. Wells, EGF receptor, Int J. Biochem. Cell. Biol. 31 (1999) 637–643.

[3] Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, Nat. Rev. Mol. Cell. Biol. 2 (2001) 127–137.

[4] S. Cohen, H. Ushiro, C. Stoscheck, M. Chinkers, A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles, J. Biol. Chem. 257 (1982) 1523–1531.

[5] Y. Yarden, J. Schlessinger, Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor, Biochemistry 26 (1987) 1443–1451.

[6] Y. Yarden, J. Schlessinger, Self-phosphorylation of epidermal growth factor receptor: evidence for a model of intermolecular allosteric activation, Biochemistry 26 (1987) 1434–1442.

[7] A.M. Honegger, R.M. Kris, A. Ullrich, J. Schlessinger, Evidence that autophosphorylation of solubilized receptors for epidermal growth factor is mediated by intermolecular cross-phosphorylation, Proc. Natl. Acad. Sci. USA 86 (1989) 925–929.

[8] A.M. Honegger, A. Schmidt, A. Ullrich, J. Schlessinger, Evidence for epidermal growth factor (EGF)-induced intermolecular autophosphorylation of the EGF receptors in living cells, Mol. Cell. Biol. 10 (1990) 4035–4044.

[9] S. Bogdan, C. Klambt, Epidermal growth factor receptor signaling, Curr. Biol. 11 (2001) R292–R295.

[10] P.O. Hackel, E. Zwick, N. Prenzel, A. Ullrich, Epidermal growth factor receptors: critical mediators of multiple receptor pathways, Curr. Opin. Cell. Biol. 11 (1999) 184–189.

[11] C. Mineo, G.N. Gill, R.G. Anderson, Regulated migration of epidermal growth factor receptor from caveolae, J. Biol. Chem. 274 (1999) 30636–30643.

[12] G. Carpenter, S. Cohen, 125I-labeled human epidermal growth factor. Binding, internalization, and degradation in human fibroblasts, J. Cell. Biol. 71 (1976) 159–171.

[13] L. Beguinot, R.M. Lyall, M.C. Willingham, I. Pastan, Down-regulation of the epidermal growth factor receptor in KB cells is due to receptor internalization and subsequent degradation in lysosomes, Proc. Natl. Acad. Sci. USA. 81 (1984) 2384–2388.

[14] A. Sorkin, C.M. Waters, Endocytosis of growth factor receptors, Bioessays 15 (1993) 375–382.

[15] H.S. Wiley, J.J. Herbst, B.J. Walsh, D.A. Lauffenburger, M.G. Rosenfeld, G.N. Gill, The role of tyrosine kinase activity in endocytosis, compartmentation, and down-regulation of the epidermal growth factor receptor, J. Biol. Chem. 266 (1991) 11083–11094.

[16] C. Lamaze, S.L. Schmid, Recruitment of epidermal growth factor receptors into coated pits requires their activated tyrosine kinase, J. Cell. Biol. 129 (1995) 47–54.

[17] M.A. Olayloye, R.M. Neve, H.A. Lane, N.E. Hyne, The ErbB signaling network: receptor heterodimerization in development and cancer, EMBO J. 19 (2000) 3159–3167.

[18] T.P. Garrett, N.M. McKern, M. Lou, T.C. Elleman, T.E. Adams, G.O. Lovrecz, H.J. Zhu, F. Walker, M.J. Frenkel, P.A. Hoyne, R.N. Jorissen, E.C. Nice, A.W. Burgess, C.W. Ward, Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha, Cell 110 (2002) 763–773.

[19] H. Ogiso, R. Ishitani, O. Nureki, S. Fukai, M. Yamanaka, J.H. Kim, K. Saito, A. Sakamoto, M. Inoue, M. Shirouzu, S. Yokoyama, Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains, Cell 110 (2002) 775–787.

[20] H.S. Cho, D.J. Leahy, Structure of the extracellular region of HER3 reveals an interdomain tether, Science 297 (2002) 1330–1333.

[21] J. Stamos, M.X. Sliwkowski, C. Eigenbrot, Structure of the EGF receptor kinase domain alone and in complex with a4-anilinoquinazoline inhibitor, J. Biol. Chem. 277 (2002) 46265–46272.

[22] A. Ullrich, L. Coussens, J.S. Hayflick, T.J. Dull, A. Gray, A.W. Tam, J. Lee, Y. Yarden, T.A. Libermann, J. Schlessinger, J. Downward, E.L.V. Mayes, N. Whittle, M.D. Waterfield, P.H. Seeburg, Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells, Nature 309 (1984) 418–425.

[23] L.J. Slieker, T.M. Martensen, M.D. Lane, Synthesis of epidermal growth factor receptor in human A431 cells. Glycosylation-dependent acquisition of ligand binding activity occurs post-translationally in the endoplasmic reticulum, J. Biol. Chem. 261 (1986) 15233–15241.

[24] T.G. Johns, E. Stockert, G. Ritter, A.A. Jungbluth, H.J. Huang, W.K. Cavenee, F.E. Smyth, C.M. Hall, N. Watson, E.C. Nice, W.J. Gullick, L.J. Old, A.W. Burgess, A.M. Scott, Novel monoclonal antibody specific for the de2-7 epidermal growth factor receptor (EGFR) that also recognizes the EGFR expressed in cells containing amplification of the EGFR gene, Int. J. Cancer. 98 (2002) 398–408.

[25] T.P. Garrett, N.M. McKern, M. Lou, M.J. Frenkel, J.D. Bentley, G.O. Lovreez, T.C. Elleman, L.J. Cosgrove, C.W. Ward, Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor, Nature 394 (1998) 395–399.

[26] T.C. Elleman, T. Domagala, N.M. McKern, M. Nerrie, B. Lonnqvist, T.E. Adams, J. Lewis, G.O. Lovrecz, P.A. Hoyne, K.M. Richards, G.J. Howlett, J. Rothacker, R.N. Jorissen, M. Lou, T.P. Garrett, A.W. Burgess, E.C. Nice, C.W. Ward, Identification of a determinant of epidermal growth factor receptor ligand-binding specificity using a truncated, high-affinity form of the ectodomain, Biochemistry 40 (2001) 8930–8939.

[27] M. Yamabhai, R.G. Anderson, Second cysteine-rich region of epidermal growth factor receptor contains targeting information for caveolae/rafts, J. Biol. Chem. 277 (2002) 24843–24846.

[28] A.C. Rigby, C.W. Grant, G.S. Shaw, Solution and solid state conformation of the human EGF receptor transmembrane region, Biochim. Biophys. Acta. 1371 (1998) 241–253.

[29] S.J. Kil, C. Carlin, EGF receptor residues leu(679), leu(680) mediate selective sorting of ligand-receptor complexes in early endosomal compartments, J. Cell. Physiol. 185 (2000) 47–60.

[30] C. He, M. Hobert, L. Friend, C. Carlin, The epidermal growth factor receptor juxtamembrane domain has multiple basolateral plasma membrane localization determinants, including a dominant signal with a polyproline core, J. Biol. Chem. 277 (2002) 38284–38293.

[31] P. Castagnino, Z. Biesova, W.T. Wong, F. Fazioli, G.N. Gill, P.P. Di Fiore, Direct binding of eps8 to the juxtamembrane domain of EGFR is phosphotyrosine- and SH2-independent, Oncogene 10 (1995) 723–729.

[32] J. Martin-Nieto, A. Villalobo, The human epidermal growth factor receptor contains a juxtamembrane calmodulin-binding site, Biochemistry 37 (1998) 227–236.
[33] H. Li, A. Villalobo, Evidence for the direct interaction between calmodulin and the human epidermal growth factor receptor, Biochem. J. 362 (2002) 499–505.
[34] S.R. Hubbard, Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog, EMBO J. 16 (1997) 5572–5581.
[35] J.C. den Hartigh, P.M. van Bergen en Henegouwen, A.J. Verkleij, J. Boonstra, The EGF receptor is an actin-binding protein, J. Cell. Biol. 119 (1992) 349–355.
[36] C.T. Kuan, C.J. Wikstrand, D.D. Bigner, EGF mutant receptor vIII as a molecular target in cancer therapy, Endocr. Relat. Cancer 8 (2001) 83–96.
[37] H.S. Huang, M. Nagane, C.K. Klingbeil, H. Lin, R. Nishikawa, X.D. Ji, C.M. Huang, G.N. Gill, H.S. Wiley, W.K. Cavenee, The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling, J. Biol. Chem. 272 (1997) 2927–2935.
[38] W. Weber, G.N. Gill, J. Spiess, Production of an epidermal growth factor receptor-related protein, Science 224 (1984) 294–297.
[39] J.V. Ilekis, B.C. Stark, B. Scoccia, Possible role of variant RNA transcripts in the regulation of epidermal growth factor receptor expression in human placenta, Mol. Reprod. Dev. 41 (1995) 149–156.
[40] J.V. Ilekis, J. Gariti, C. Niederberger, B. Scoccia, Expression of a truncated epidermal growth factor receptor-like protein (TEGFR) in ovarian cancer, Gynecol. Oncol. 65 (1997) 36–41.
[41] J.L. Reiter, N.J. Maihle, A 1.8 kb alternative transcript from the human epidermal growth factor receptor gene encodes a truncated form of the receptor, Nucleic Acids. Res. 24 (1996) 4050–4056.
[42] L.C. Groenen, E.C. Nice, A.W. Burgess, Structure-function relationships for the EGF/TGF-alpha family of mitogens, Growth Factors 11 (1994) 235–257.
[43] E.C. Nice, T. Domagala, L. Fabri, M. Nerriea, F. Walker, R.N. Jorissen, A.W. Burgess, D.F. Cui, Y.S. Zhang, Rapid microscale enzymic semisynthesis of epidermal growth factor (EGF) analogues, Growth Factors 20 (2002) 71–80.
[44] L. Strachan, J.G. Murison, R.L. Prestidge, M.A. Sleeman, J.D. Watson, K.D. Kumble, Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily, J. Biol. Chem. 276 (2001) 18265–18271.
[45] S.A. Thompson, A. Harris, D. Hoang, M. Ferrer, G.R. Johnson, COOH-terminal extended recombinant amphiregulin with bioactivity comparable with naturally derived growth factor, J. Biol. Chem. 271 (1996) 17927–17931.
[46] B. Neelam, A. Richter, S.G. Chamberlin, S.M. Puddicombe, L. Wood, M.B. Murray, K. Nandagopal, S.K. Niyogi, D.E. Davies, Structure-function studies of ligand-induced epidermal growth factor receptor dimerization, Biochemistry 37 (1998) 4884–4891.
[47] J.T. Jones, R.W. Akita, M.X. Sliwkowski, Binding specificities and affinities of egf domains for ErbB receptors, FEBS Lett. 447 (1999) 227–231.
[48] D. Harari, E. Tzahar, J. Romano, M. Shelly, J.H. Pierce, G.C. Andrews, Y. Yarden, Neuregulin-4: a novel growth factor that acts through the ErbB-4 receptor tyrosine kinase, Oncogene 18 (1999) 2681–2689.
[49] D. Kohda, M. Odaka, I. Lax, H. Kawasaki, K. Suzuki, A. Ullrich, J. Schlessinger, F. Inagaki, A 40-kDa epidermal growth factor/transforming growth factor alpha-binding domain produced by limited proteolysis of the extracellular domain of the epidermal growth factor receptor, J. Biol. Chem. 268 (1993) 1976–1981.
[50] M.A. Lemmon, Z. Bu, J.E. Ladbury, M. Zhou, D. Pinchasi, I. Lax, D.M. Engelman, J. Schlessinger, Two EGF molecules contribute additively to stabilization of the EGFR dimer, EMBO J. 16 (1997) 281–294.
[51] J.H. Kim, K. Saito, S. Yokoyama, Chimeric receptor analyses of the interactions of the ectodomains of ErbB-1 with epidermal growth factor and of those of ErbB-4 with neuregulin, Eur. J. Biochem. 269 (2002) 2323–2329.
[52] E. Singer, R. Landgraf, T. Horan, D. Slamon, D. Eisenberg, Identification of a heregulin binding site in HER3 extracellular domain, J. Biol. Chem. 276 (2001) 44266–44274.
[53] E.G. Barbacci, B.C. Guarino, J.G. Stroh, D.H. Singleton, K.J. Rosnack, J.D. Moyer, G.C. Andrews, The structural basis for the specificity of epidermal growth factor and heregulin binding, J. Biol. Chem. 270 (1995) 9585–9589.
[54] A. Harris, M. Adler, J. Brink, R. Lin, M. Foehr, M. Ferrer, B.C. Langton-Webster, R.N. Harkins, S.A. Thompson, Homologue scanning mutagenesis of heregulin reveals receptor specific binding epitopes, Biochem. Biophys. Res. Commun. 251 (1998) 220–224.
[55] C. Stortelers, C. Souriau, E. van Liempt, M.L. van de Poll, E.J. van Zoelen, Role of the N-terminus of epidermal growth factor in ErbB-2/ErbB-3 binding studied by phage display, Biochemistry 41 (2002) 8732–8741.
[56] M. Adler, S.A. Thompson, The solution structure of heregulin-alpha and a N-terminal mutant with suppressed activity, Biochem. Biophys. Res. Commun. 256 (1999) 156–161.
[57] R.R. Beerli, N.E. Hynes, Epidermal growth factor-related peptides activate distinct subsets of ErbB receptors and differ in their biological activities, J. Biol. Chem. 271 (1996) 6071–6076.
[58] C.R. Lin, W.S. Chen, C.S. Lazar, C.D. Carpenter, G.N. Gill, R.M. Evans, M.G. Rosenfeld, Protein kinase C phosphorylation at Thr 654 of the unoccupied EGF receptor and EGF binding regulate functional receptor loss by independent mechanisms, Cell 44 (1986) 839–848.
[59] M. Boni-Schnetzler, P.F. Pilch, Mechanism of epidermal growth factor receptor autophosphorylation and high-affinity binding, Proc. Natl. Acad. Sci. USA 84 (1987) 7832–7836.
[60] D.R. Hurwitz, S.L. Emanuel, M.H. Nathan, N. Sarver, A. Ullrich, S. Felder, I. Lax, J. Schlessinger, EGF induces increased ligand binding affinity and dimerization of soluble epidermal growth factor (EGF) receptor extracellular domain, J. Biol. Chem. 266 (1991) 22035–22043.
[61] A. Sorokin, M.A. Lemmon, A. Ullrich, J. Schlessinger, Stabilization of an active dimeric form of the epidermal growth factor receptor by introduction of an inter-receptor disulfide bond, J. Biol. Chem. 269 (1994) 9752–9759.
[62] M. Martin-Fernandez, D.T. Clarke, M.J. Tobin, S.V. Jones, G.R. Jones, Preformed oligomeric epidermal growth factor receptors undergo an ectodomain structure change during signaling, Biophys. J. 82 (2002) 2415–2427.
[63] R. Prywes, E. Livneh, A. Ullrich, J. Schlessinger, Mutations in the cytoplasmic domain of EGF receptor affect EGF binding and receptor internalization, EMBO J. 5 (1986) 2179–2190.
[64] K.J. Fowler, F. Walker, W. Alexander, M.L. Hibbs, E.C. Nice, R.M. Bohmer, G.B. Mann, C. Thumwood, R. Maglitto, J.A. Danks, A mutation in the epidermal growth factor receptor in waved-2 mice has a profound effect on receptor biochemistry that results in impaired lactation, Proc. Natl. Acad. Sci. USA 92 (1995) 1465–1469.
[65] L.C. Groenen, F. Walker, A.W. Burgess, H.R. Treutlein, A model for the activation of the epidermal growth factor receptor kinase involvement of an asymmetric dimer? Biochemistry 36 (1997) 3826–3836.
[66] F. Walker, M.L. Hibbs, H.H. Zhang, L.J. Gonez, A.W. Burgess, Biochemical characterization of mutant EGF receptors expressed in the hemopoietic cell line BaF/3, Growth Factors 16 (1998) 53–67.
[67] M.A. Van der Heyden, M. Nievers, A.J. Verkleij, J. Boonstra, P.M. van Bergen en Henegouwen, Identification of an intracellular domain of the EGF receptor required for high-affinity binding of EGF, FEBS Lett. 410 (1997) 265–268.
[68] F. Walker, A.W. Burgess, Reconstitution of the high affinity epidermal growth factor receptor on cell-free membranes after

transmodulation by platelet-derived growth factor, J. Biol. Chem. 266 (1991) 2746–2752.
[69] M.R. Holbrook, L.L. Slakey, D.J. Gross, Thermodynamic mixing of molecular states of the epidermal growth factor receptor modulates macroscopic ligand binding affinity, Biochem. J. 352 (Pt 1) (2000) 99–108.
[70] M. Odaka, D. Kohda, I. Lax, J. Schlessinger, F. Inagaki, Ligand-binding enhances the affinity of dimerization of the extracellular domain of the epidermal growth factor receptor, J. Biochem. (Tokyo) 122 (1997) 116–121.
[71] T. Domagala, N. Konstantopoulos, F. Smyth, R.N. Jorissen, L. Fabri, D. Geleick, I. Lax, J. Schlessinger, W. Sawyer, G.J. Howlett, A.W. Burgess, E.C. Nice, Stoichiometry, kinetic and binding analysis of the interaction between epidermal growth factor (EGF) and the extracellular domain of the EGF receptor, Growth Factors 18 (2000) 11–29.
[72] Y. Sako, S. Minoghchi, T. Yanagida, Single-molecule imaging of EGFR signalling on the surface of living cells, Nat. Cell. Biol. 2 (2000) 168–172.
[73] T. Moriki, H. Maruyama, I.N. Maruyama, Activation of preformed EGF receptor dimers by ligand-induced rotation of the transmembrane domain, J. Mol. Biol. 311 (2001) 1011–1026.
[74] A. Berezov, J. Chen, Q. Liu, H.T. Zhang, M.I. Greene, R. Murali, Disabling receptor ensembles with rationally designed interface peptidomimetics, J. Biol. Chem. 277 (2002) 28330–28339.
[75] J. Schlessinger, Dynamics of hormone receptors on cell membrane, Ann. NY Acad. Sci. 366 (1981) 274–284.
[76] A. Chantry, The kinase domain and membrane localization determine intracellular interactions between epidermal growth factor receptors, J. Biol. Chem. 270 (1995) 3068–3073.
[77] K.G. Tanner, J. Kyte, Dimerization of the extracellular domain of the receptor for epidermal growth factor containing the membrane-spanning segment in response to treatment with epidermal growth factor, J. Biol. Chem. 274 (1999) 35985–35990.
[78] D. Graus-Porta, R.R. Beerli, J.M. Daly, N.E. Hynes, ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling, EMBO J. 16 (1997) 1647–1655.
[79] D. Karunagaran, E. Tzahar, R.R. Beerli, X. Chen, D. Graus-Porta, B.J. Ratzkin, R. Seger, N.E. Hynes, Y. Yarden, ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer, EMBO J. 15 (1996) 254–264.
[80] A.E. Lenferink, R. Pinkas-Kramarski, M.L. van de Poll, M.J. van Vugt, L.N. Klapper, E. Tzahar, H. Waterman, M. Sela, E.J. van Zoelen, Y. Yarden, Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers, EMBO J. 17 (1998) 3385–3397.
[81] G.C. Huang, X. Ouyang, R.J. Epstein, Proxy activation of protein ErbB2 by heterologous ligands implies a heterotetrameric mode of receptor tyrosine kinase interaction, Biochem. J. 331 (Pt 1) (1998) 113–119.
[82] K.M. Ferguson, P.J. Darling, M.J. Mohan, T.L. Macatee, M.A. Lemmon, Extracellular domains drive homo- but not hetero-dimerization of erbB receptors, EMBO J. 19 (2000) 4632–4643.
[83] D.C. Gamett, G. Pearson, R.A. Cerione, I. Friedberg, Secondary dimerization between members of the epidermal growth factor receptor family, J. Biol. Chem. 272 (1997) 12052–12056.
[84] L.E. Johannessen, K.E. Haugen, A.C. Ostvold, E. Stang, I.H. Madshus, Heterodimerization of the epidermal-growth-factor (EGF) receptor and ErbB2 and the affinity of EGF binding are regulated by different mechanisms, Biochem. J. 356 (2001) 87–96.
[85] Y. Saito, J. Haendeler, Y. Hojo, K. Yamamoto, B.C. Berk, Receptor heterodimerization: essential mechanism for platelet-derived growth factor-induced epidermal growth factor receptor transactivation, Mol. Cell. Biol. 21 (2001) 6387–6394.
[86] T.W. Gadella Jr., T.M. Jovin, Oligomerization of epidermal growth factor receptors on A431 cells studied by time-resolved fluorescence imaging microscopy. A stereochemical model for tyrosine kinase receptor activation, J. Cell. Biol. 129 (1995) 1543–1558.
[87] X. Yu, K.D. Sharma, T. Takahashi, R. Iwamoto, E. Mekada, Ligand-independent dimer formation of epidermal growth factor receptor (EGFR) is a step separable from ligand-induced EGFR signaling, Mol. Biol. Cell. 13 (2002) 2547–2557.
[88] P.A. Humphrey, A.J. Wong, B. Vogelstein, H.S. Friedman, M.H. Werner, D.D. Bigner, S.H. Bigner, Amplification and expression of the epidermal growth factor receptor gene in human glioma xenografts, Cancer Res. 48 (1988) 2231–2238.
[89] H. Yamazaki, Y. Fukui, Y. Ueyama, N. Tamaoki, T. Kawamoto, S. Taniguchi, M. Shibuya, Amplification of the structurally and functionally altered epidermal growth factor receptor gene (c-erbB) in human brain tumors, Mol. Cell. Biol. 8 (1988) 1816–1820.
[90] L.T. Malden, U. Novak, A.H. Kaye, A.W. Burgess, Selective amplification of the cytoplasmic domain of the epidermal growth factor receptor gene in glioblastoma multiforme, Cancer Res. 48 (1988) 2711–2714.
[91] M.M. Kwatra, D.D. Bigner, J.A. Cohn, The ligand binding domain of the epidermal growth factor receptor is not required for receptor dimerization, Biochim. Biophys. Acta 1134 (1992) 178–181.
[92] A.J. Ekstrand, N. Longo, M.L. Hamid, J.J. Olson, L. Liu, V.P. Collins, C.D. James, Functional characterization of an EGF receptor with a truncated extracellular domain expressed in glioblastomas with EGFR gene amplification, Oncogene 9 (1994) 2313–2320.
[93] H. Fernandes, S. Cohen, S. Bishayee, Glycosylation-induced conformational modification positively regulates receptor-receptor association: a study with an aberrant epidermal growth factor receptor (EGFRvIII/DeltaEGFR) expressed in cancer cells, J. Biol. Chem. 276 (2001) 5375–5383.
[94] F. Canals, Signal transmission by epidermal growth factor receptor: coincidence of activation and dimerization, Biochemistry 31 (1992) 4493–4501.
[95] J.M. Sherrill, Insufficiency of self-phosphorylation for the activation of epidermal growth factor receptor, Biochemistry 36 (1997) 5677–5684.
[96] S.R. Hubbard, M. Mohammadi, J. Schlessinger, Autoregulatory mechanisms in protein-tyrosine kinases, J. Biol. Chem. 273 (1998) 11987–11990.
[97] D.A. Tice, J.S. Biscardi, A.L. Nickles, S.J. Parsons, Mechanism of biological synergy between cellular Src and epidermal growth factor receptor, Proc. Natl. Acad. Sci. USA 96 (1999) 1415–1420.
[98] H. Yamaguchi, W.A. Hendrickson, Structural basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation, Nature 384 (1996) 484–489.
[99] C.L. Arteaga, T.T. Ramsey, L.K. Shawver, C.A. Guyer, Unliganded epidermal growth factor receptor dimerization induced by direct interaction of quinazolines with the ATP binding site, J. Biol. Chem. 272 (1997) 23247–23254.
[100] R.B. Lichtner, A. Menrad, A. Sommer, U. Klar, M.R. Schneider, Signaling-inactive epidermal growth factor receptor/ligand complexes in intact carcinoma cells by quinazoline tyrosine kinase inhibitors, Cancer Res. 61 (2001) 5790–5795.
[101] R.B. Montgomery, Antagonistic and agonistic effects of quinazoline tyrosine kinase inhibitors on mutant EGF receptor function, Int. J. Cancer 101 (2002) 111–117.
[102] M. Huse, J. Kuriyan, The conformational plasticity of protein kinases, Cell 109 (2002) 275–282.
[103] G. Ge, J. Wu, Y. Wang, Q. Lin, Activation mechanism of solubilized epidermal growth factor receptor tyrosine kinase, Biochem. Biophys. Res. Commun. 290 (2002) 914–920.
[104] R.J. Daly, Take your partners, please--signal diversification by the erbB family of receptor tyrosine kinases, Growth Factors 16 (1999) 255–263.
[105] T.B. Deb, L. Su, L. Wong, E. Bonvini, A. Wells, M. David, G.R. Johnson, Epidermal growth factor (EGF) receptor kinase-independent signaling by EGF, J. Biol. Chem. 276 (2001) 15554–15560.

[106] J.A. Ewald, K.J. Coker, J.O. Price, J.V. Staros, C.A. Guyer, Stimulation of mitogenic pathways through kinase-impaired mutants of the epidermal growth factor receptor, Exp. Cell. Res. 268 (2001) 262–273.
[107] T. Yamauchi, K. Ueki, K. Tobe, H. Tamemoto, N. Sekine, M. Wada, M. Honjo, M. Takahashi, T. Takahashi, H. Hirai, T. Tushima, Y. Akanuma, T. Fujita, I. Komuro, Y. Yazaki, T. Kadowaki, Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone, Nature 390 (1997) 91–96.
[108] T. Fukazawa, S. Miyake, V. Band, H. Band, Tyrosine phosphorylation of Cb1 upon epidermal growth factor (EGF) stimulation and its association with EGF receptor and downstream signaling proteins, J. Biol. Chem. 271 (1996) 14554–14559.
[109] M. Wheeler, J. Domin, Recruitment of the class II phosphoinositide 3-kinase C2beta to the epidermal growth factor receptor: role of Grb2, Mol. Cell. Biol. 21 (2001) 6660–6667.
[110] M.T. Kloth, K.K. Laughlin, J.S. Biscardi, J.L. Boerner, S.J. Parsons, C.M. Silva, STAT5b, a mediator of synergism between c-Src and the EGF receptor, J. Biol. Chem. 278 (2003) 1671–1679.
[111] N.J. Hellyer, K. Cheng, J.G. Koland, ErbB3 (HER3) interaction with the p85 regulatory subunit of phosphoinositide 3-kinase, Biochem. J. 333 (Pt 3) (1998) 757–763.
[112] Z. Galcheva-Gargova, K.N. Konstantinov, I.H. Wu, F.G. Klier, T. Barrett, R.J. Davis, Binding of zinc finger protein ZPR1 to the epidermal growth factor receptor, Science 272 (1996) 1795–1797.
[113] L. Xia, L. Wang, A.S. Chung, S.S. Ivanov, M.Y. Ling, A.M. Dragoi, A. Platt, T.M. Gilmer, X.Y. Fu, Y.E. Chin, Identification of both positive and negative domains within the epidermal growth factor receptor COOH-terminal region for signal transducer and activator of transcription (STAT) activation, J. Biol. Chem. 277 (2002) 30716–30723.
[114] M.A. Olayioye, I. Beuvink, K. Horsch, J.M. Daly, N.E. Hynes, ErbB receptor-induced activation of stat transcription factors is mediated by Src tyrosine kinases, J. Biol. Chem. 274 (1999) 17209–17218.
[115] F. Walker, A. Kato, L.J. Gonez, M.L. Hibbs, N. Pouliot, A. Levitzki, A.W. Burgess, Activation of the Ras/mitogen-activated protein kinase pathway by kinase-defective epidermal growth factor receptors results in cell survival but not proliferation, Mol. Cell. Biol. 18 (1998) 7192–7204.
[116] G.M. Walton, W.S. Chen, M.G. Rosenfeld, G.N. Gill, Analysis of deletions of the carboxyl terminus of the epidermal growth factor receptor reveals self-phosphorylation at tyrosine 992 and enhanced in vivo tyrosine phosphorylation of cell substrates, J. Biol. Chem. 265 (1990) 1750–1754.
[117] N. Gotoh, A. Tojo, K. Muroya, Y. Hashimoto, S. Hattori, S. Nakamura, T. Takenawa, Y. Yazaki, M. Shibuya, Epidermal growth factor-receptor mutant lacking the autophosphorylation sites induces phosphorylation of Shc protein and Shc-Grb2/ASH association and retains mitogenic activity, Proc. Natl. Acad. Sci. USA 91 (1994) 167–171.
[118] L. Wong, T.B. Deb, S.A. Thompson, A. Wells, G.R. Johnson, A differential requirement for the COOH-terminal region of the epidermal growth factor (EGF) receptor in amphiregulin and EGF mitogenic signaling, J. Biol. Chem. 274 (1999) 8900–8909.
[119] D.R. Stover, M. Becker, J. Liebetanz, N.B. Lydon, Src phosphorylation of the epidermal growth factor receptor at novel sites mediates receptor interaction with Src and P85 alpha, J. Biol. Chem. 270 (1995) 15591–15597.
[120] E.J. Lowenstein, R.J. Daly, A.G. Batzer, W. Li, B. Margolis, R. Lammers, A. Ullrich, E.Y. Skolnik, D. Bar-Sagi, J. Schlessinger, The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling, Cell 70 (1992) 431–442.
[121] A.G. Batzer, D. Rotin, J.M. Urena, E.Y. Skolnik, J. Schlessinger, Hierarchy of binding sites for Grb2 and Shc on the epidermal growth factor receptor, Mol. Cell. Biol. 14 (1994) 5192–5201.
[122] T. Sasaoka, W.J. Langlois, J.W. Leitner, B. Draznin, J.M. Olefsky, The signaling pathway coupling epidermal growth factor receptors to activation of p21ras, J. Biol. Chem. 269 (1994) 32621–32625.
[123] K. Sakaguchi, Y. Okabayashi, Y. Kido, S. Kimura, Y. Matsumura, K. Inushima, M. Kasuga, Shc phosphotyrosine-binding domain dominantly interacts with epidermal growth factor receptors and mediates Ras activation in intact cells, Mol. Endocrinol. 12 (1998) 536–543.
[124] A. Hashimoto, M. Kurosaki, N. Gotoh, M. Shibuya, T. Kurosaki, Shc regulates epidermal growth factor-induced activation of the JNK signaling pathway, J. Biol. Chem. 274 (1999) 20139–20143.
[125] B. Hallberg, S.I. Rayter, J. Downward, Interaction of Ras and Raf in intact mammalian cells upon extracellular stimulation, J. Biol. Chem. 269 (1994) 3913–3916.
[126] G.L. Johnson, R.R. Vaillancourt, Sequential protein kinase reactions controlling cell growth and differentiation, Curr. Opin. Cell. Biol. 6 (1994) 230–238.
[127] W.J. Langlois, T. Sasaoka, A.R. Saltiel, J.M. Olefsky, Negative feedback regulation and desensitization of insulin- and epidermal growth factor-stimulated p21ras activation, J. Biol. Chem. 270 (1995) 25320–25323.
[128] T. Jun, O. Gjoerup, T.M. Roberts, Tangled webs: evidence of cross-talk between c-Raf-1 and Akt, Sci. STKE. 1999 (1999) E1.
[129] C. Liebmann, Regulation of MAP kinase activity by peptide receptor signalling pathway: paradigms of multiplicity, Cell. Signal. 13 (2001) 777–785.
[130] J. Pouyssegur, V. Volmat, P. Lenormand, Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling, Biochem. Pharmacol. 64 (2002) 755–763.
[131] H. Meisner, M.P. Czech, Coupling of the proto-oncogene product c-Cb1 to the epidermal growth factor receptor, J. Biol. Chem. 270 (1995) 25332–25335.
[132] D.D. Schlaepfer, C.R. Hauck, D.J. Sieg, Signaling through focal adhesion kinase, Prog. Biophys. Mol. Biol. 71 (1999) 435–478.
[133] X.X. Xu, T. Yi, B. Tang, J.D. Lambeth, Disabled-2 (Dab2) is an SH3 domain-binding partner of Grb2, Oncogene 16 (1998) 1561–1569.
[134] P. De Sepulveda, K. Okkenhaug, J.L. Rose, R.G. Hawley, P. Dubreuil, R. Rottapel, Socs1 binds to multiple signalling proteins and suppresses steel factor-dependent proliferation, EMBO J. 18 (1999) 904–915.
[135] S.L. Harmer, A.L. DeFranco, The src homology domain 2-containing inositol phosphatase SHIP forms a ternary complex with Shc and Grb2 in antigen receptor-stimulated B lymphocytes, J. Biol. Chem. 274 (1999) 12183–12191.
[136] G. Pelicci, L. Lanfrancone, A.E. Salcini, A. Romano, S. Mele, B.M. Grazia, O. Segatto, P.P. Di Fiore, P.G. Pelicci, Constitutive phosphorylation of Shc proteins in human tumors, Oncogene 11 (1995) 899–907.
[137] M. Pomerance, M.C. Multon, F. Parker, C. Venot, J.P. Blondeau, B. Tocque, F. Schweighoffer, Grb2 interaction with MEK-kinase 1 is involved in regulation of Jun-kinase activities in response to epidermal growth factor, J. Biol. Chem. 273 (1998) 24301–24304.
[138] Y. Xu, D.F. Guo, M. Davidson, T. Inagami, G. Carpenter, Interaction of the adaptor protein Shc and the adhesion molecule cadherin, J. Biol. Chem. 272 (1997) 13463–13466.
[139] M. Damelin, P.A. Silver, Mapping interactions between nuclear transport factors in living cells reveals pathways through the nuclear pore complex, Mol. Cell. 5 (2000) 133–140.
[140] A.P. Belsches, M.D. Haskell, S.J. Parsons, Role of c-Src tyrosine kinase in EGF-induced mitogenesis, Front. Biosci. 2 (1997) d501–d518.
[141] D.K. Luttrell, L.M. Luttrell, S.J. Parsons, Augmented mitogenic responsiveness to epidermal growth factor in murine fibroblasts that overexpress pp60c-src, Mol. Cell. Biol. 8 (1988) 497–501.
[142] M.C. Maa, T.H. Leu, D.J. McCarley, R.C. Schatzman, S.J. Parsons, Potentiation of epidermal growth factor receptor-mediated oncogen-

esis by c-Src: implications for the etiology of multiple human cancers, Proc. Natl. Acad. Sci. USA 92 (1995) 6981–6985.
[143] S. Roche, M. Koegl, M.V. Barone, M.F. Roussel, S.A. Courtneidge, DNA synthesis induced by some but not all growth factors requires Src family protein tyrosine kinases, Mol. Cell. Biol. 15 (1995) 1102–1109.
[144] M.V. Barone, S.A. Courtneidge, Myc but not Fos rescue of PDGF signalling block caused by kinase-inactive Src [see comments], Nature 378 (1995) 509–512.
[145] R. Karni, R. Jove, A. Levitzki, Inhibition of pp60c-Src reduces Bcl-XL expression and reverses the transformed phenotype of cells overexpressing EGF and HER-2 receptors, Oncogene 18 (1999) 4654–4662.
[146] N. Osherov, A. Levitzki, Epidermal-growth-factor-dependent activation of the src-family kinases, Eur. J. Biochem. 225 (1994) 1047–1053.
[147] W. Mao, R. Irby, D. Coppola, L. Fu, M. Wloch, J. Turner, H. Yu, R. Garcia, R. Jove, T.J. Yeatman, Activation of c-Src by receptor tyrosine kinases in human colon cancer cells with high metastatic potential, Oncogene 15 (1997) 3083–3090.
[148] J.S. Biscardi, M.C. Maa, D.A. Tice, M.E. Cox, T.H. Leu, S.J. Parsons, c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function, J. Biol. Chem. 274 (1999) 8335–8343.
[149] C.R. Lombardo, T.G. Consler, D.B. Kassel, In vitro phosphorylation of the epidermal growth factor receptor autophosphorylation domain by c-src: identification of phosphorylation sites and c-src SH2 domain binding sites, Biochemistry 34 (1995) 16456–16466.
[150] K. Sato, A. Sato, M. Aoto, Y. Fukami, c-Src phosphorylates epidermal growth factor receptor on tyrosine 845, Biochem. Biophys. Res. Commun. 215 (1995) 1078–1087.
[151] R.L. Kendall, R.Z. Rutledge, X. Mao, A.J. Tebben, R.W. Hungate, K.A. Thomas, Vascular endothelial growth factor receptor KDR tyrosine kinase activity is increased by autophosphorylation of two activation loop tyrosine residues, J. Biol. Chem. 274 (1999) 6453–6460.
[152] M.E. Cunningham, L.A. Greene, A function-structure model for NGF-activated TRK, EMBO J. 17 (1998) 7282–7293.
[153] N. Gotoh, A. Tojo, M. Hino, Y. Yakazi, M. Shibuya, A highly conserved tyrosine residue at codon 845 within the kinase domain is not required for the transforming activity of human epidermal growth factor receptor, Biochem. Biophys. Res. Commun. 186 (1992) 768–774.
[154] J.D. Wright, C.W. Reuter, M.J. Weber, An incomplete program of cellular tyrosine phosphorylations induced by kinase-defective epidermal growth factor receptors, J. Biol. Chem. 270 (1995) 12085–12093.
[155] K. Sato, N. Gotoh, T. Otsuki, M. Kakumoto, M. Aoto, A.A. Tokmakov, M. Shibuya, Y. Fukami, Tyrosine residues 239 and 240 of Shc are phosphatidylinositol 4,5- bisphosphate-dependent phosphorylation sites by c-Src, Biochem. Biophys. Res. Commun. 240 (1997) 399–404.
[156] J.H. Chang, S. Gill, J. Settleman, S.J. Parsons, c-Src regulates the simultaneous rearrangement of actin cytoskeleton, p190RhoGAP, and p120RasGAP following epidermal growth factor stimulation, J. Cell. Biol. 130 (1995) 355–368.
[157] R.W. Roof, M.D. Haskell, B.D. Dukes, N. Sherman, M. Kinter, S.J. Parsons, Phosphotyrosine (p-Tyr)-dependent and -independent mechanisms of p190 RhoGAP-p120 RasGAP interaction: Tyr 1105 of p190, a substrate for c- Src, is the sole p-Tyr mediator of complex formation, Mol. Cell. Biol. 18 (1998) 7052–7063.
[158] Y. Nojima, T. Mimura, N. Morino, K. Hamasaki, H. Furuya, R. Sakai, T. Nakamoto, Y. Yazaki, H. Hirai, Tyrosine phosphorylation of p130Cas in cell adhesion and transformation, Hum. Cell 9 (1996) 169–174.
[159] C. Huang, J. Liu, C.C. Haudenschild, X. Zhan, The role of tyrosine phosphorylation of cortactin in the locomotion of endothelial cells, J. Biol. Chem. 273 (1998) 25770–25776.
[160] O. Lohi, V.P. Lehto, EAST, a novel EGF receptor substrate, associates with focal adhesions and actin fibers, FEBS Lett. 436 (1998) 419–423.
[161] C. Provenzano, R. Gallo, R. Carbone, P.P. Di Fiore, G. Falcone, L. Castellani, S. Alema, Eps8, a tyrosine kinase substrate, is recruited to the cell cortex and dynamic F-actin upon cytoskeleton remodeling, Exp. Cell. Res. 242 (1998) 186–200.
[162] S.E. Shoelson, M. Sivaraja, K.P. Williams, P. Hu, J. Schlessinger, M.A. Weiss, Specific phosphopeptide binding regulates a conformational change in the PI 3-kinase SH2 domain associated with enzyme activation, EMBO J. 12 (1993) 795–802.
[163] J.E. Damell Jr., STATs and gene regulation, Science 277 (1997) 1630–1635.
[164] J.N. Ihle, T. Nosaka, W. Thierfelder, F.W. Quelle, K. Shimoda, Jaks and Stats in cytokine signaling, Stem Cells 15 Suppl 1 (1997) 105–111.
[165] W.J. Leonard, Role of Jak kinases and STATs in cytokine signal transduction, Int. J. Hematol. 73 (2001) 271–277.
[166] O.K. Park, T.S. Schaefer, D. Nathans, In vitro activation of Stat3 by epidermal growth factor receptor kinase, Proc. Natl. Acad. Sci. USA 93 (1996) 13704–13708.
[167] M. David, L. Wong, R. Flavell, S.A. Thompson, A. Wells, A.C. Larner, G.R. Johnson, STAT activation by epidermal growth factor (EGF) and amphiregulin. Requirement for the EGF receptor kinase but not for tyrosine phosphorylation sites or JAK1, J. Biol. Chem. 271 (1996) 9185–9188.
[168] D.W. Leaman, S. Pisharody, T.W. Flickinger, M.A. Commane, J. Schlessinger, I.M. Kerr, D.E. Levy, G.R. Stark, Roles of JAKs in activation of STATs and stimulation of c-fos gene expression by epidermal growth factor, Mol. Cell. Biol. 16 (1996) 369–375.
[169] M.G. Houle, S. Bourgoin, Regulation of phospholipase D by phosphorylation-dependent mechanisms, Biochim. Biophys. Acta 1439 (1999) 135–149.
[170] R. Slaaby, T. Jensen, H.S. Hansen, M.A. Frohman, K. Seedorf, PLD2 complexes with the EGF receptor and undergoes tyrosine phosphorylation at a single site upon agonist stimulation, J. Biol. Chem. 273 (1998) 33722–33727.
[171] S.M. Hernandez-Sotomayor, G. Carpenter, Non-catalytic activation of phospholipase C-gamma 1 in vitro by epidermal growth factor receptor, Biochem. J. 293 (Pt 2) (1993) 507–511.
[172] A. Kamat, G. Carpenter, Phospholipase C-gamma1: regulation of enzyme function and role in growth factor-dependent signal transduction, Cytokine Growth Factor Rev. 8 (1997) 109–117.
[173] A. Chattopadhyay, M. Vecchi, Q. Ji, R. Mernaugh, G. Carpenter, The role of individual SH2 domains in mediating association of phospholipase C-gamma 1 with the activated EGF receptor, J. Biol. Chem. 274 (1999) 26091–26097.
[174] M. Wahl, S. Nishibe, J.W. Kim, H. Kim, S.G. Rhee, G. Carpenter, Identification of two epidermal growth factor-sensitive tyrosine phosphorylation sites of phospholipase C-gamma in intact HSC-1 cells, J. Biol. Chem. 265 (1990) 3944–3948.
[175] F. Hofer, R. Berdeaux, G.S. Martin, Ras-independent activation of Ral by a Ca(2+)-dependent pathway, Curr. Biol. 8 (1998) 839–842.
[176] L. Sun, G. Carpenter, Epidermal growth factor activation of NF-kappaB is mediated through IkappaBalpha degradation and intracellular free calcium, Oncogene 16 (1998) 2095–2102.
[177] R. Marais, Y. Light, C. Mason, H. Paterson, M.F. Olson, C.J. Marshall, Requirement of Ras-GTP-Raf complexes for activation of Raf-1 by protein kinase C [published erratum appears in Science 1998 May 15;280(5366):987], Science 280 (1998) 109–112.
[178] M. McClellan, P. Kievit, N. Auersperg, K. Rodland, Regulation of proliferation and apoptosis by epidermal growth factor and protein kinase C in human ovarian surface epithelial cells, Exp. Cell. Res. 246 (1999) 471–479.
[179] Y.S. Liaw, P.C. Yang, C.J. Yu, S.H. Kuo, K.T. Luh, Y.J. Lin, M.L. Wu, PKC activation is required by EGF-stimulated Na(+)-H+

exchanger in human pleural mesothelial cells, Am. J. Physiol. 274 (1998) L665–L672.
[180] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655–1657.
[181] S. Djordjevic, P.C. Driscoll, Structural insight into substrate specificity and regulatory mechanisms of phosphoinositide 3-kinases, Trends. Biochem. Sci. 27 (2002) 426–432.
[182] C.L. Carpenter, K.R. Auger, M. Chanudhuri, M. Yoakim, B. Schaffhausen, S. Shoelson, L.C. Cantley, Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit, J. Biol. Chem. 268 (1993) 9478–9483.
[183] H.H. Kim, S.L. Sierke, J.G. Koland, Epidermal growth factor-dependent association of phosphatidylinositol 3-kinase with the erbB3 gene product, J. Biol. Chem. 269 (1994) 24747–24755.
[184] T.G. Ram, S.P. Ethier, Phosphatidylinositol 3-kinase recruitment by p185erbB-2 and erbB-3 is potently induced by neu differentiation factor/heregulin during mitogenesis and is constitutively elevated in growth factor-independent breast carcinoma cells with c-erbB-2 gene amplification, Cell. Growth. Differ. 7 (1996) 551–561.
[185] P.F. Jones, T. Jakubowicz, F.J. Pitossi, F. Maurer, B.A. Hemmings, Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily, Proc. Natl. Acad. Sci. USA 88 (1991) 4171–4175.
[186] K.M. Nicholson, N.G. Anderson, The protein kinase B/Akt signalling pathway in human malignancy, Cell. Signal. 14 (2002) 381–395.
[187] M.E. Pacold, S. Suire, O. Perisic, S. Lara-Gonzalez, C.T. Davis, E.H. Walker, P.T. Hawkins, L. Stephens, J.F. Eccleston, R.L. Williams, Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma, Cell 103 (2000) 931–943.
[188] S. Cohen, Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal, J. Biol. Chem. 237 (1962) 1555–1562.
[189] H. Gregory, Isolation and structure of urogastrone and its relationship to epidermal growth factor, Nature 257 (1975) 325–327.
[190] J.E. de Larco, G.J. Todaro, Growth factors from murine sarcoma virus-transformed cells, Proc. Natl. Acad. Sci. USA 75 (1978) 4001–4005.
[191] J.M. Smith, M.B. Sporn, A.B. Roberts, R. Derynck, M.E. Winkler, H. Gregory, Human transforming growth factor-alpha causes precocious eyelid opening in newborn mice, Nature 315 (1985) 515–516.
[192] R.T. Topham, D.J.J. Chiego, V.H. Gattone, D.A. Hinton, R.M. Klein, The effect of epidermal growth factor on neonatal incisor differentiation in the mouse, Dev. Biol. 124 (1987) 532–543.
[193] G. Calamandrei, E. Alleva, Epidermal growth factor has both growth-promoting and growth-inhibiting effects on physical and neurobehavioral development of neonatal mice, Brain Res. 477 (1989) 1–6.
[194] J.L. Smart, S.V. da, L.R. Malheiros, F.J. Paumgartten, R.F. Massey, Epidermal growth factor advances some aspects of development but retards others in both rats and hamsters, J. Dev. Physiol. 11 (1989) 153–158.
[195] R. Vassar, E. Fuchs, Transgenic mice provide new insights into the role of TGF-alpha during epidermal development and differentiation, Genes Dev. 5 (1991) 714–727.
[196] A.M. Dominey, X.J. Wang, L.E.J. King, L.B. Nanney, T.A. Gagne, K. Sellheyer, D.S. Bundman, M.A. Longley, J.A. Rothnagel, D.A. Greenhalgh, Targeted overexpression of transforming growth factor alpha in the epidermis of transgenic mice elicits hyperplasia, hyperkeratosis, and spontaneous, squamous papillomas, Cell Growth Differ. 4 (1993) 1071–1082.
[197] P.W. Cook, M. Piepkorn, C.H. Clegg, G.D. Plowman, J.M. DeMay, J.R. Brown, M.R. Pittelkow, Transgenic expression of the human amphiregulin gene induces a psoriasis-like phenotype, J. Clin. Invest. 100 (1997) 2286–2294.
[198] D.A. Lowden, G.W. Lindemann, G. Merlino, B.D. Barash, J.P. Calvet, V.H. Gattone, Renal cysts in transgenic mice expressing transforming growth factor-alpha [see comments], J. Lab. Clin. Med. 124 (1994) 386–394.
[199] V.H. Gattone, K.A. Kuenstler, G.W. Lindemann, X. Lu, B.D.J. Cowley, C.A. Rankin, J.P. Calvet, Renal expression of a transforming growth factor-alpha transgene accelerates the progression of inherited, slowly progressive polycystic kidney disease in the mouse, J. Lab. Clin. Med. 127 (1996) 214–222.
[200] B.R. Davies, A.M. Platt-Higgins, G. Schmidt, P.S. Rudland, Development of hyperplasias, preneoplasias, and mammary tumors in MMTV-c-erbB-2 and MMTV-TGF alpha transgenic rats, Am. J. Pathol. 155 (1999) 303–314.
[201] E.P. Sandgren, J.A. Schroeder, T.H. Qui, R.D. Palmiter, R.L. Brinster, D.C. Lee, Inhibition of mammary gland involution is associated with transforming growth factor alpha but not c-myc-induced tumorigenesis in transgenic mice, Cancer Res. 55 (1995) 3915–3927.
[202] D.J. Hehir, G. McGreal, W.O. Kirwan, W. Kealy, M.P. Brady, c-myc oncogene expression: a marker for females at risk of breast carcinoma, J. Surg. Oncol. 54 (1993) 207–209.
[203] D.W. Visscher, T. Wallis, S. Awussah, A. Mohamed, J.D. Crissman, Evaluation of MYC and chromosome 8 copy number in breast carcinoma by interphase cytogenetics, Genes Chromosomes Cancer 18 (1997) 1–7.
[204] I. Bieche, R. Lidereau, Genetic alterations in breast cancer, Genes Chromosomes Cancer 14 (1995) 227–251.
[205] L.T. Amundadottir, S.J. Nass, G.J. Berchem, M.D. Johnson, R.B. Dickson, Cooperation of TGF alpha and c-Myc in mouse mammary tumorigenesis: coordinated stimulation of growth and suppression of apoptosis, Oncogene 13 (1996) 757–765.
[206] H. Murakami, N.D. Sanderson, P. Nagy, P.A. Marino, G. Merlino, S.S. Thorgeirsson, Transgenic mouse model for synergistic effects of nuclear oncogenes and growth factors in tumorigenesis: interaction of c-myc and transforming growth factor alpha in hepatic oncogenesis, Cancer Res. 53 (1993) 1719–1723.
[207] S.S. Thorgeirsson, E. Santoni-Rugiu, Interaction of c-myc with transforming growth factor alpha and hepatocyte growth factor in hepatocarcinogenesis, Mutat. Res. 376 (1997) 221–234.
[208] E. Santoni-Rugiu, M.R. Jensen, S.S. Thorgeirsson, Disruption of the pRb/E2F pathway and inhibition of apoptosis are major oncogenic events in liver constitutively expressing c-myc and transforming growth factor alpha, Cancer Res. 58 (1998) 123–134.
[209] L.M. Sargent, N.D. Sanderson, S.S. Thorgeirsson, Ploidy and karyotypic alterations associated with early events in the development of hepatocarcinogenesis in transgenic mice harboring c-myc and transforming growth factor alpha transgenes, Cancer Res. 56 (1996) 2137–2142.
[210] J. Schlegel, A. Merdes, G. Stumm, F.K. Albert, M. Forsting, N. Hynes, M. Kiessling, Amplification of the epidermal-growth-factor-receptor gene correlates with different growth behaviour in human glioblastoma, Int. J. Cancer 56 (1994) 72–77.
[211] D.K. Moscatello, M. Holgado-Madruga, A.K. Godwin, G. Ramirez, G. Gunn, P.W. Zoltick, J.A. Biegel, R.L. Hayes, A.J. Wong, Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors, Cancer Res. 55 (1995) 5536–5539.
[212] N. Sugawa, A.J. Ekstrand, C.D. James, V.P. Collins, Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas, Proc. Natl. Acad. Sci. USA 87 (1990) 8602–8606.
[213] A.J. Ekstrand, N. Sugawa, C.D. James, V.P. Collins, Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails, Proc. Natl. Acad. Sci. USA 89 (1992) 4309–4313.
[214] R. Nishikawa, X.D. Ji, R.C. Harmon, C.S. Lazar, G.N. Gill, W.K. Cavenee, H.J. Huang, A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity, Proc. Natl. Acad. Sci. USA 91 (1994) 7727–7731.
[215] M. Nagane, F. Coufal, H. Lin, O. Bogler, W.K. Cavenee, H.J. Huang, A common mutant epidermal growth factor receptor confers enhanced

tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis, Cancer Res. 56 (1996) 5079–5086.
[216] E.C. Holland, W.P. Hively, R.A. DePinho, H.E. Varmus, A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice, Genes Dev. 12 (1998) 3675–3685.
[217] R.M. Bachoo, E.A. Maher, K.L. Ligon, N.E. Sharpless, S.S. Chan, M.J. You, Y. Tang, J. DeFrances, E. Stover, R. Weissleder, D.H. Rowitch, D.N. Louis, R.A. DePinho, Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis, Cancer Cell. 1 (2002) 269–277.
[218] S. Okamoto, T. Oka, Evidence for physiological function of epidermal growth factor: pregestational sialoadenectomy of mice decreases milk production and increases offspring mortality during lactation period, Proc. Natl. Acad. Sci. USA 81 (1984) 6059–6063.
[219] O. Tsutsumi, Y. Kubota, T. Oka, Effect of sialoadenectomy, treatment with epidermal growth factor (EGF) antiserum and replacement of EGF on the epidermis in mice, J. Endocrinol. 113 (1987) 193–197.
[220] N.C. Luetteke, T.H. Qiu, R.L. Peiffer, P. Oliver, O. Smithies, D.C. Lee, TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice, Cell 73 (1993) 263–278.
[221] G.B. Mann, K.J. Fowler, A. Gabriel, E.C. Nice, R.L. Williams, A.R. Dunn, Mice with a null mutation of the TGF alpha gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation, Cell 73 (1993) 249–261.
[222] M. Blum, A null mutation in TGF-alpha leads to a reduction in midbrain dopaminergic neurons in the substantia nigra, Nat. Neurosci. 1 (1998) 374–377.
[223] N.C. Luetteke, T.H. Qiu, S.E. Fenton, K.L. Troyer, R.F. Riedel, A. Chang, D.C. Lee, Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development, Development 126 (1999) 2739–2750.
[224] D.W. Threadgill, A.A. Dlugosz, L.A. Hansen, T. Tennenbaum, U. Lichti, D. Yee, C. LaMantia, T. Mourton, K. Herrup, R.C. Harris, Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype, Science 269 (1995) 230–234.
[225] M. Sibilia, E.F. Wagner, Strain-dependent epithelial defects in mice lacking the EGF receptor [published erratum appears in Science 1995 Aug 18;269(5226):909], Science 269 (1995) 234–238.
[226] P.J. Miettinen, J.E. Berger, J. Meneses, Y. Phung, R.A. Pedersen, Z. Werb, R. Derynck, Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor, Nature 376 (1995) 337–341.
[227] N.C. Luetteke, H.K. Phillips, T.H. Qiu, N.G. Copeland, H.S. Earp, N.A. Jenkins, D.C. Lee, The mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase, Genes Dev. 8 (1994) 399–413.
[228] W. Xie, A.J. Paterson, E. Chin, L.M. Nabell, J.E. Kudlow, Targeted expression of a dominant negative epidermal growth factor receptor in the mammary gland of transgenic mice inhibits pubertal mammary duct development, Mol. Endocrinol. 11 (1997) 1766–1781.
[229] J.F. Wiesen, P. Young, Z. Werb, G.R. Cunha, Signaling through the stromal epidermal growth factor receptor is necessary for mammary ductal development, Development 126 (1999) 335–344.
[230] D.A. Lauffenburger, A.F. Horwitz, Cell migration: a physically integrated molecular process, Cell 84 (1996) 359–369.
[231] T.J. Mitchison, L.P. Cramer, Actin-based cell motility and cell locomotion, Cell 84 (1996) 371–379.
[232] I. Kaverina, O. Krylyshkina, J.V. Small, Regulation of substrate adhesion dynamics during cell motility, Int. J. Biochem. Cell. Biol. 34 (2002) 746–761.
[233] C.C. Kumar, Signaling by integrin receptors, Oncogene 17 (1998) 1365–1373.
[234] S. Dedhar, Cell-substrate interactions and signaling through ILK, Curr. Opin. Cell. Biol. 12 (2000) 250–256.
[235] F.G. Giancotti, E. Ruoslahti, Integrin signaling, Science 285 (1999) 1028–1032.
[236] K. Matsumoto, B.L. Ziober, C.C. Yao, R.H. Kramer, Growth factor regulation of integrin-mediated cell motility, Cancer Metastasis Rev. 14 (1995) 205–217.
[237] M. Iijima, Y.E. Huang, P. Devreotes, Temporal and spatial regulation of chemotaxis, Dev. Cell. 3 (2002) 469–478.
[238] A. Wells, K. Gupta, P. Chang, S. Swindle, A. Glading, H. Shiraha, Epidermal growth factor receptor-mediated motility in fibroblasts, Microsc. Res. Tech. 43 (1998) 395–411.
[239] L.G. Hudson, L.J. McCawley, Contributions of the epidermal growth factor receptor to keratinocyte motility, Microsc. Res. Tech. 43 (1998) 444–455.
[240] A. Wells, Tumor invasion: role of growth factor-induced cell motility, Adv. Cancer Res. 78 (2000) 31–101.
[241] J. Kassis, D.A. Lauffenburger, T. Turner, A. Wells, Tumor invasion as dysregulated cell motility, Semin. Cancer Biol. 11 (2001) 105–117.
[242] P. Chen, H. Xie, M.C. Sekar, K. Gupta, A. Wells, Epidermal growth factor receptor-mediated cell motility: phospholipase C activity is required, but mitogen-activated protein kinase activity is not sufficient for induced cell movement, J. Cell. Biol. 127 (1994) 847–857.
[243] P. Chen, K. Gupta, A. Wells, Cell movement elicited by epidermal growth factor receptor requires kinase and autophosphorylation but is separable from mitogenesis, J. Cell. Biol. 124 (1994) 547–555.
[244] P. Chen, J.E. Murphy-Ullrich, A. Wells, A role for gelsolin in actuating epidermal growth factor receptor-mediated cell motility, J. Cell. Biol. 134 (1996) 689–698.
[245] F.N. van Leeuwen, S. van Delft, H.E. Kain, R.A. van der Kammen, J.G. Collard, Rac regulates phosphorylation of the myosin-II heavy chain, actinomyosin disassembly and cell spreading, Nat. Cell. Biol. 1 (1999) 242–248.
[246] P.E. Hughes, M.W. Renshaw, M. Pfaff, J. Forsyth, V.M. Keivens, M.A. Schwartz, M.H. Ginsberg, Suppression of integrin activation: a novel function of a Ras/Raf-initiated MAP kinase pathway, Cell 88 (1997) 521–530.
[247] H. Xie, M.A. Pallero, K. Gupta, P. Chang, M.F. Ware, W. Witke, D.J. Kwiatkowski, D.A. Lauffenburger, J.E. Murphy-Ullrich, A. Wells, EGF receptor regulation of cell motility: EGF induces disassembly of focal adhesions independently of the motility-associated PLC gamma signaling pathway, J. Cell. Sci. 111 (Pt 5) (1998) 615–624.
[248] M.P. Sheetz, D.P. Felsenfeld, C.G. Galbraith, Cell migration: regulation of force on extracellular-matrix-integrin complexes, Trends Cell. Biol. 8 (1998) 51–54.
[249] A. Huttenlocher, S.P. Palecek, Q. Lu, W. Zhang, R.L. Mellgren, D.A. Lauffenburger, M.H. Ginsberg, A.F. Horwitz, Regulation of cell migration by the calcium-dependent protease calpain, J. Biol. Chem. 272 (1997) 32719–32722.
[250] H. Shiraha, A. Glading, K. Gupta, A. Wells, IP-10 inhibits epidermal growth factor-induced motility by decreasing epidermal growth factor receptor-mediated calpain activity, J. Cell. Biol. 146 (1999) 243–254.
[251] S.P. Palecek, J.C. Loftus, M.H. Ginsberg, D.A. Lauffenburger, A.F. Horwitz, Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness, Nature 385 (1997) 537–540.
[252] A. Glading, D.A. Lauffenburger, A. Wells, Cutting to the chase: calpain proteases in cell motility, Trends Cell. Biol. 12 (2002) 46–54.
[253] J. Lee, A. Ishihara, G. Oxford, B. Johnson, K. Jacobson, Regulation of cell movement is mediated by stretch-activated calcium channels, Nature 400 (1999) 382–386.
[254] R.L. Klemke, S. Cai, A.L. Giannini, P.J. Gallagher, P. de Lanerolle, D.A. Cheresh, Regulation of cell motility by mitogen-activated protein kinase, J. Cell. Biol. 137 (1997) 481–492.
[255] A.R. Bresnick, Molecular mechanisms of nonmuscle myosin-II regulation, Curr. Opin. Cell. Biol. 11 (1999) 26–33.

[256] E. Zamir, B.Z. Katz, S. Aota, K.M. Yamada, B. Geiger, Z. Kam, Molecular diversity of cell-matrix adhesions, J. Cell. Sci. 112 (Pt 11) (1999) 1655–1669.
[257] B.S. Verbeek, S.S. Adriaansen-Slot, T.M. Vroom, T. Beckers, G. Rijksen, Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts, FEBS Lett. 425 (1998) 145–150.
[258] D. Theodorescu, K.R. Laderoute, K.M. Gulding, Epidermal growth factor receptor-regulated human bladder cancer motility is in part a phosphatidylinositol 3-kinase-mediated process, Cell. Growth Differ. 9 (1998) 919–928.
[259] C.J. Wu, Z. Chen, A. Ullrich, M.I. Greene, D.M. O'Rourke, Inhibition of EGFR-mediated phosphoinositide-3-OH kinase (PI3-K) signaling and glioblastoma phenotype by signal-regulatory proteins (SIRPs), Oncogene 19 (2000) 3999–4010.
[260] A. Hall, Rho GTPases and the actin cytoskeleton, Science 279 (1998) 509–514.
[261] P. Aspenstrom, Effectors for the Rho GTPases, Curr. Opin. Cell. Biol. 11 (1999) 95–102.
[262] S.M. Schoenwaelder, K. Burridge, Bidirectional signaling between the cytoskeleton and integrins, Curr. Opin. Cell. Biol. 11 (1999) 274–286.
[263] C.D. Nobes, A. Hall, Rho GTPases control polarity, protrusion, and adhesion during cell movement, J. Cell. Biol. 144 (1999) 1235–1244.
[264] A. Malliri, M. Symons, R.F. Hennigan, A.F. Hurlstone, R.F. Lamb, T. Wheeler, B.W. Ozanne, The transcription factor AP-1 is required for EGF-induced activation of rho-like GTPases, cytoskeletal rearrangements, motility, and in vitro invasion of A43I cells, J. Cell. Biol. 143 (1998) 1087–1099.
[265] P.J. Keely, J.K. Westwick, I.P. Whitehead, C.J. Der, L.V. Parise, Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K, Nature 390 (1997) 632–636.
[266] P. Keely, L. Parise, R. Juliano, Integrins and GTPases in tumour cell growth, motility and invasion, Trends Cell. Biol. 8 (1998) 101–106.
[267] L.E. Rameh, L.C. Cantley, The role of phosphoinositide 3-kinase lipid products in cell function, J. Biol. Chem. 274 (1999) 8347–8350.
[268] S.J. Leevers, B. Vanhaesebroeck, M.D. Waterfield, Signalling through phosphoinositide 3-kinases: the lipids take centre stage, Curr. Opin. Cell. Biol. 11 (1999) 219–225.
[269] P.T. Hawkins, A. Eguinoa, R.G. Qiu, D. Stokoe, F.T. Cooke, R. Walters, S. Wennstrom, L. Claesson-Welsh, T. Evans, M. Symons, L. Stephens, PDGF stimulates an increase in GTP-Rac via activation of phosphoinositide 3-kinase, Curr. Biol. 5 (1995) 393–403.
[270] J. Han, K. Luby-Phelps, B. Das, X. Shu, Y. Xia, R.D. Mosteller, U.M. Krishna, J.R. Falck, M.A. White, D. Broek, Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanosine triphosphatases by Vav, Science 279 (1998) 558–560.
[271] J.A. Greenwood, A.B. Theibert, G.D. Prestwich, J.E. Murphy-Ullrich, Restructuring of focal adhesion plaques by PI 3-kinase. Regulation by PtdIns (3,4,5)-p(3) binding to alpha-actinin, J. Cell. Biol. 150 (2000) 627–642.
[272] E. Piccolo, P.F. Innominato, M.A. Mariggio, T. Maffucci, S. Iacobelli, M. Falasca, The mechanism involved in the regulation of phospholipase Cgamma1 activity in cell migration, Oncogene 21 (2002) 6520–6529.
[273] O.D. Weiner, Rac activation: P-Rex1—a convergence point for PIP(3) and Gbetagamma? Curr. Biol. 12 (2002) R429–R431.
[274] A.C. Porter, R.R. Vaillancourt, Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis, Oncogene 17 (1998) 1343–1352.
[275] S. Corvera, M.P. Czech, Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction, Trends Cell. Biol. 8 (1998) 442–446.
[276] I.S. Nathke, C.L. Adams, P. Polakis, J.H. Sellin, W.J. Nelson, The adenomatous polyposis coli tumor suppressor protein localizes to plasma membrane sites involved in active cell migration, J. Cell. Biol. 134 (1996) 165–179.
[277] J.I. Fenton, M.S. Wolff, M.W. Orth, N.G. Hord, Membrane-type matrix metalloproteinases mediate curcumin-induced cell migration in non-tumorigenic colon epithelial cells differing in Apc genotype, Carcinogenesis 23 (2002) 1065–1070.
[278] M.F. Ware, A. Wells, D.A. Lauffenburger, Epidermal growth factor alters fibroblast migration speed and directional persistence reciprocally and in a matrix-dependent manner, J. Cell. Sci. 111 (Pt 16) (1998) 2423–2432.
[279] J. Gu, M. Tamura, R. Pankov, E.H. Danen, T. Takino, K. Matsumoto, K.M. Yamada, Shc and FAK differentially regulate cell motility and directionality modulated by PTEN, J. Cell. Biol. 146 (1999) 389–403.
[280] M. Zhao, A. Dick, J.V. Forrester, C.D. McCaig, Electric field-directed cell motility involves up-regulated expression and asymmetric redistribution of the epidermal growth factor receptors and is enhanced by fibronectin and laminin, Mol. Biol. Cell. 10 (1999) 1259–1276.
[281] K.S. Fang, E. Ionides, G. Oster, R. Nuccitelli, R.R. Isseroff, Epidermal growth factor receptor relocalization and kinase activity are necessary for directional migration of keratinocytes in DC electric fields, J. Cell. Sci. 112 (Pt 12) (1999) 1967–1978.
[282] M. Zhao, J. Pu, J.V. Forrester, C.D. McCaig, Membrane lipids, EGF receptors, and intracellular signals colocalize and are polarized in epithelial cells moving directionally in a physiological electric field, FASEB J. 16 (2002) 857–859.
[283] J. Li, M.L. Lin, G.J. Wiepz, A.G. Guadarrama, P.J. Bertics, Integrin-mediated migration of murine B82L fibroblasts is dependent on the expression of an intact epidermal growth factor receptor, J. Biol. Chem. 274 (1999) 11209–11219.
[284] T. Okutani, Y. Okabayashi, Y. Kido, Y. Sugimoto, K. Sakaguchi, K. Matuoka, T. Takenawa, M. Kasuga, Grb2/Ash binds directly to tyrosines 1068 and 1086 and indirectly to tyrosine 1148 of activated human epidermal growth factor receptors in intact cells, J. Biol. Chem. 269 (1994) 31310–31314.
[285] J.H. McCarty, The Nck SH2/SH3 adaptor protein: a regulator of multiple intracellular signal transduction events, Bioessays 20 (1998) 913–921.
[286] Y. Hashimoto, H. Katayama, E. Kiyokawa, S. Ota, T. Kurata, N. Gotoh, N. Otsuka, M. Shibata, M. Matsuda, Phosphorylation of CrkII adaptor protein at tyrosine 221 by epidermal growth factor receptor, J. Biol. Chem. 273 (1998) 17186–17191.
[287] N. Jones, D.J. Dumont, Recruitment of Dok-R to the EGF receptor through its PTB domain is required for attenuation of Erk MAP kinase activation, Curr. Biol. 9 (1999) 1057–1060.
[288] J. Serth, W. Weber, M. Frech, A. Wittinghofer, A. Pingoud, Binding of the H-ras p21 GTPase activating protein by the activated epidermal growth factor receptor leads to inhibition of the p21 GTPase activity in vitro, Biochemistry 31 (1992) 6361–6365.
[289] K.L. Milarski, G. Zhu, C.G. Pearl, D.J. McNamara, E.M. Dobrusin, D. MacLean, A. Thieme-Sefler, Z.Y. Zhang, T. Sawyer, S.J. Decker, Sequence specificity in recognition of the epidermal growth factor receptor by protein tyrosine phosphatase 1B, J. Biol. Chem. 268 (1993) 23634–23639.
[290] H. Keilhack, T. Tenev, E. Nyakatura, J. Godovac-Zimmermann, L. Nielsen, K. Seedorf, F.D. Bohmer, Phosphotyrosine 1173 mediates binding of the protein-tyrosine phosphatase SHP-1 to the epidermal growth factor receptor and attenuation of receptor signaling, J. Biol. Chem. 273 (1998) 24839–24846.
[291] G. Zhu, S.J. Decker, D. MacLean, D.J. McNamara, J. Singh, T.K. Sawyer, A.R. Saltiel, Sequence specificity in the recognition of the epidermal growth factor receptor by the ab1 Src homology 2 domain, Oncogene 9 (1994) 1379–1385.
[292] P.J. Kraulis, MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures, J. Appl. Crystallogr. 24 (1991) 946–950.
[293] E.A. Merritt, D.J. Bacon, Raster3D: photorealistic molecular graphics, Methods Enzymol. 277 (1997) 505–524.