The EMBO Medal for 1992 has been awarded to Dr Carl-Henrik Heldin of the Ludwig Institute for Cancer Research, Biomedical Centre, Uppsala, Sweden. Dr Heldin receives the Medal for his work on platelet derived growth factor (PDGF) about which he writes in the review beginning on the facing page.

The Medal is sponsored by the following companies:

AKZO, Amersham, ASTRA, Becton-Dickinson, Boehringer Ingelheim, Boehringer Mannheim, Carlsberg Bryggerierne, Ciba-Geigy AG, F. Hoffman-La Roche AG, Sandoz AG, EMBL, Farmitalia, Glaxo, ICI, Kontron, LKB, Merieux, NOVO Industri A/S, Pharmacia, Sanofi, Sclavo, Senetek, Tuborgfondet.

The EMBO Journal vol.11 no.12 pp.4251 – 4259, 1992

Structural and functional studies on platelet-derived growth factor

Carl-Henrik Heldin

Ludwig Institute for Cancer Research, Biomedial Centre, Box 595, S-751 24 Uppsala, Sweden

Introduction

In 1975 I started a Ph.D. thesis project at the University of Uppsala with Åke Wasteson and Bengt Westermark as teachers. The theme of the project was studies of the mechanisms that control growth of cells in culture. The idea was that knowledge about such control mechanisms in normal cells would be valuable in order to understand the lack of growth control of cancer cells. We were particularly interested in the role of growth factors, i.e. factors that stimulate cells to divide, in the control of cell growth. Bengt and Åke had observed that human platelets contained a growth promoting activity for glia-like cells in culture (Westermark and Wasteson, 1976). Growth promoting activities for smooth muscle cells (Ross *et al.*, 1974) and 3T3 cells (Kohler and Lipton, 1974) had also been reported to be present in platelets and it seemed likely that they represented the same molecule. At this time, knowledge about the structural and functional properties of growth factors was very limited, and only one, epidermal growth factor (EGF) (Carpenter and Cohen, 1979), was available in pure form.

Since the growth factor in platelets, later designated platelet-derived growth factor (PDGF), seemed to be the major activity in calf serum for promoting the growth of several cell types, it was obviously important to purify and further characterize this factor. In addition, Bengt and Åke had found that a human osteosarcoma cell line, U-2OS, secreted a growth factor into the culture medium (Westermark and Wasteson, 1975). This observation was interesting as it fitted nicely into the concept that over-production of growth factors by malignant cells could be involved in loss of growth control by self-stimulation, later called autocrine growth stimulation by Sporn and Todaro (1980). The aim of my thesis project was to purify and characterize PDGF and the factor produced by the osteosarcoma cells, later designated ODGF.

Purification of PDGF

We set out to purify PDGF from lysates of outdated platelets collected from neighbouring hospitals using the protein purification techniques of the 1970s and without much appreciation of the difficulties that lay ahead of us. PDGF proved to be a very sticky molecule with both hydrophobic and cationic properties, which resulted in low recoveries in most purification steps. Moreover, the scarcity of starting material was a severely limiting factor. The situation improved considerably in 1978 when we got access to large quantities of human platelet material through the courtesy of the Finnish Red Cross in Helsinki, as a by-product of their production of interferon from human leukocytes. Later on we received additional material from the Department of Virology in Uppsala, when they also started production of interferon. The first small amounts of pure PDGF were obtained soon thereafter in our laboratory (Heldin *et al.*, 1979) as well as by Antoniades *et al.* (1979). Protocols for the production of larger quantities of PDGF were subsequently worked out (Antoniades, 1981; Deuel *et al.*, 1991; Heldin *et al.*, 1981a; Raines and Ross, 1982). In the routine preparation procedure that we established, ~100 μg of pure PDGF was obtained from the platelets derived from 200 litres of human blood (reviewed in Heldin *et al.*, 1987). Eventually we had enough material for a thorough structural and functional characterization of PDGF. Moreover, our freezers gradually filled with side-fractions from the various chromatography steps. These fractions would become very useful for Kohei Miyazono when he later joined our laboratory, as starting material for the purification of two other growth regulatory factors, the latent form of transforming growth factor-β (Miyazono *et al.*, 1988), and platelet-derived endothelial cell growth factor (Miyazono *et al.*, 1987), a factor with angiogenic activity (Ishikawa *et al.*, 1989) which recently was found to have thymidine phosphorylase activity (Usuki *et al.*, 1992). These studies initiated ongoing lines of research in our laboratory. However, in the interest of conformity, this review will focus on PDGF. It represents the evolution of the PDGF field of research seen from my own perspective; for more balanced reviews see Heldin and Westermark (1990) and Raines *et al.* (1990).

Initial characterization of PDGF revealed that it is a 30 kDa dimeric protein consisting of two different polypeptide chains connected by disulfide bonds. The two polypeptide chains were of similar size and co-eluted in most chromatography systems, but could be separated by a high resolution reversed phase HPLC; the two chains were named A and B after their order of elution in this chromatography (Johnsson *et al.*, 1982).

PDGF is structurally similar to the *sis* oncogene product

In order to obtain information about the amino acid sequence of PDGF we, and the group of Tom Deuel, collaborated with Mike Waterfield who had in his laboratory one of the few gas phase sequencers available at this time. The result of the sequencing of the A- and B-chains of PDGF, obtained during the spring of 1983, went beyond our expectations; the two chains were found to be homologous, and the B-chain was almost identical to a part of $p28^{sis}$, the transforming protein and simian sarcoma virus (SSV). This finding was

exciting because it provided a link between growth factors and oncogene products and suggested a mechanism whereby oncogene products transform cells, i.e. by subversion of the mitogenic pathway of growth factors. The spring of 1983 was hectic; we tried to obtain further evidence for the involvement of an autocrine mechanism in the transformation of SSV-infected cells. However, these attempts had to be postponed because on May 27, Antoniades and Hunkapiller (1983) published a partial amino acid sequence of PDGF. There was no mention of the similarity with the $p28^{sis}$ sequence; the *sis* sequence, which had been published in February 1983 (Devare *et al.*, 1983) was apparently not in the database at the time when Antoniades and Hunkapiller submitted their paper. Now the sequences of both PDGF and *sis* were in the public domain and we realized that we should hurry the publication of our findings. A paper was prepared and submitted to *Nature*; it appeared in print only three weeks after submission (Waterfield *et al.*, 1983). At about the same time, the homology was described by Doolittle *et al.* (1983) in a paper in *Science* which was also published about three weeks after submission.

During the following years, evidence accumulated in our group as well as in other groups in support of the notion that SSV transformed cells by production of a PDGF-like growth factor which acted in an autocrine manner (reviewed in Westermark *et al.*, 1987). The observations in our own laboratory included evidence that SSV-transformed cells in culture produce a growth factor that is immunologically related to PDGF (Johnsson *et al.*, 1985a), and that SSV-transformed cells were phenotypically normalized after addition of agents that prevent the interaction between PDGF and its receptors, such as PDGF antibodies (Johnsson *et al.*, 1985b) and suramin (Betsholtz *et al.*, 1986b). SSV-transformation was shown not to immortalize human fibroblasts, but to provide only an efficient autocrine growth stimulation (Johnsson *et al.*, 1986). The finding of a homology between the *sis* oncogene product and the B-chain of PDGF was rapidly followed by additional observations demonstrating structural and functional similarities between other oncogene products and growth factors, their receptors or components of their intracellular signal transduction pathways. Together, these observations provided support for the hypothesis that oncogenes transform cells by subverting the mitogenic pathway of growth factors (Figure 1) (Heldin and Westermark, 1984). It is no exaggeration to say that we had been lucky. Our studies on PDGF were initiated based on the notion that knowledge about the normal mechanism for growth control could be useful for understanding the molecular mechanism behind the loss of growth control in cancer. Of the numerous possible growth factors, we happened to choose to study one of the few that has been isolated as an oncogene product, and, in fact, the only one whose gene has been transduced as a retroviral oncogene.

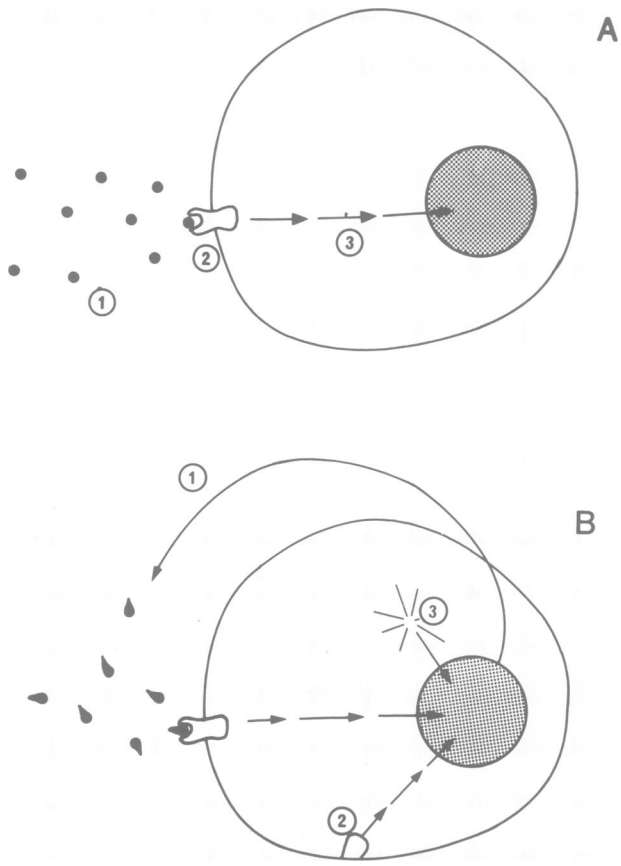

Fig. 1. Mitogenic stimulation in normal and transformed cells. (**A**) Schematic representation of the mitogenic pathway of normal cells. The growth factor (1) interacts with a cell membrane receptor (2) and activates an intracellular messenger system that transmits the mitogenic signal to the nucleus (3). (**B**) Schematic illustration of possible perturbations of the mitogenic pathway in transformed cells. The transformed cell might constitutively produce growth factors that activate endogenous receptors and stimulate growth in an autocrine manner, as occurs in SSV-transformed cells (1). Alternatively, proteins that mimic the activity of an activated growth factor receptor (2), or ones that mimic the activity of regulatory components along the intracellular messenger system (3) might be produced. (Taken from Heldin and Westermark, 1984.)

Different isoforms of PDGF

Another example of serendipity in science came when we obtained further information about the structural and functional properties of osteosarcoma cell-derived growth factor (ODGF). As we struggled with the purification of the factor, it became more and more clear that it was similar to PDGF. In 1980, we reported that ODGF was a dimeric molecule with a size similar to PDGF, had similar chromatographic properties to PDGF, and was recognized by antibodies against PDGF (Heldin *et al.*, 1980). Yet there were subtle differences between the two factors, e.g. PDGF was always a more potent growth factor than ODGF. An explanation for these observations came when we obtained enough material for amino acid sequencing of ODGF; only A-chain sequence was obtained and no B-chain sequence. We were subsequently able to show that ODGF was in fact a homodimer of the PDGF A-chain (Heldin *et al.*, 1986). Subsequent analysis of the PDGF that we routinely purified from human platelets revealed that it was a mixture of 70% PDGF-AB and 30% PDGF-BB (Hammacher *et al.*, 1988a). However, PDGF-AA is also present in human platelets (Hart *et al.*, 1990), and was therefore most probably lost in our purification procedure. Thus, the purification of the two seemingly unrelated factors, PDGF and ODGF, in the end yielded the different isoforms of PDGF. A molecular explanation for the subtle differences in functional activities of the osteosarcoma-derived PDGF-AA and PDGF-AB/-BB from platelets would come later with the analysis of the

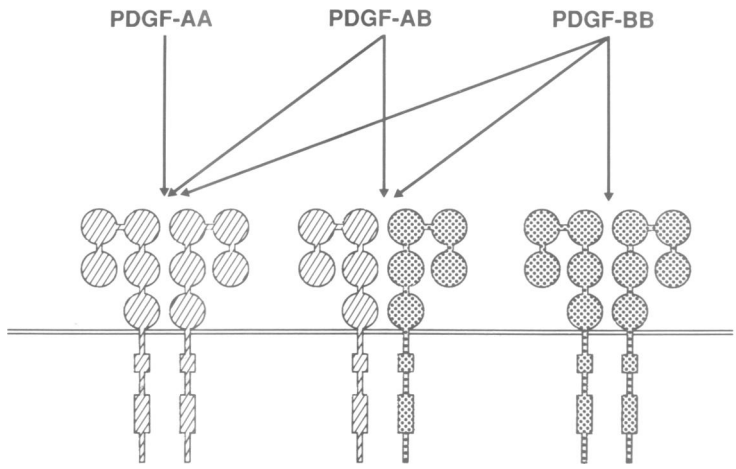

Fig. 2. Schematic illustration of the binding of the different isoforms of PDGF to homo- and heterodimeric complexes of PDGF receptors. The α-receptor (hatched) and β-receptor (dotted) are drawn to indicate that they each contain five extracellular immunoglobulin-like domains and an intracellular split tyrosine kinase. (Taken from Heldin and Westermark, 1990.)

receptor binding specificities of the different PDGF isoforms (see below).

PDGF receptors

In parallel to the studies on PDGF, we were actively trying to learn more about the molecular mechanism whereby PDGF exerts its mitogenic effect. Specific PDGF receptors were demonstrated by us (Heldin *et al.*, 1981b) and by other groups (Bowen-Pope and Ross, 1982; Huang *et al.*, 1982; Singh *et al.*, 1982; Williams *et al.*, 1982). The PDGF receptor was shown to become internalized and down-regulated after ligand binding (Heldin *et al.*, 1982), and affinity labelling techniques were used to determine its size [~185 kDa (Glenn *et al.*, 1982; Heldin *et al.*, 1983)]. Following the demonstration that the EGF receptor is a protein tyrosine kinase (Ushiro and Cohen, 1980), we were able to show that the PDGF receptor similarly has a ligand-stimulatable intrinsic protein tyrosine kinase activity (Ek and Heldin, 1982; Ek *et al.*, 1982; Heldin *et al.*, 1983) and that stimulation by PDGF led to the phosphorylation of specific cytoplasmic substrates (Ek and Heldin, 1984). These findings were of considerable interest as they suggested that protein tyrosine phosphorylation might be a general mechanism for growth stimulation.

The cloning of the PDGF receptor was an obvious, but difficult, goal at this time. In order to obtain amino acid sequence information, we worked out a large scale purification procedure for the receptor using as starting material porcine uterus, the pig organ that was richest in PDGF receptor. The purification eventually succeeded (Rönnstrand *et al.*, 1987), but the mouse receptor was cloned more rapidly by Yarden *et al.* (1986).

There were, however, further questions to resolve regarding PDGF receptors. A comparison of the functional activities of a glioma-derived PDGF-AA with those of PDGF-AB purified from human platelets revealed differences; both isoforms stimulated mitogenicity in human foreskin fibroblasts, albeit with low potency in the case of PDGF-AA, but only PDGF-AB was able to stimulate chemotaxis and actin reorganization resulting in the formation of circular membrane ruffles (Nistér *et al.*, 1988a). A possible interpretation of these observations was that more than one type of PDGF receptor might exist, whose binding specificities for the different PDGF isoforms were different. The presence of a second PDGF receptor was subsequently shown by us (Heldin *et al.*, 1988) and others (Hart *et al.*, 1988). This new receptor bound all isoforms of PDGF with high affinity and was subsequently designated the α-receptor (initially, the term A-type receptor was also used). The PDGF receptor that had previously been identified, purified and cloned was designated the β-receptor (B-type receptor); it was found to bind only PDGF-BB with high affinity.

Our capacity for cDNA cloning was significantly improved when Lena Claesson-Welsh joined our laboratory in 1986. A strategy to clone the α-receptor was devised using a human glioma cell line which expressed only α-receptors (Claesson-Welsh *et al.*, 1989a) as a source of mRNA. Using a previously cloned human PDGF β-receptor probe (Claesson-Welsh *et al.*, 1988) and low stringency conditions, a cDNA for the human α-receptor was subsequently obtained (Claesson-Welsh *et al.*, 1989b); the same sequence was also obtained by Matsui *et al.* (1989). Comparison of the sequence with that of the human β-receptor revealed that the two receptors are structurally similar; each consists of an extracellular part with five immunoglobulin-like domains and an intracellular part containing a kinase domain that includes a characteristic inserted sequence without homology to other kinases.

Signal transduction

The intrinsic kinase activity of the PDGF β-receptor was, not surprisingly, found to be essential for the mediation of cell growth (Escobedo *et al.*, 1988) and chemotaxis (Westermark *et al.*, 1990). However, a kinase negative mutant of the receptor was able to become internalized and degraded after ligand-binding, albeit more slowly than the wild-type receptor (Sorkin *et al.*, 1991).

How does the binding of PDGF to the extracellular part of the receptors activate their intracellular kinases? The fact that the PDGF molecule is dimeric suggested that it might simultaneously bind to two receptors and thus cause receptor

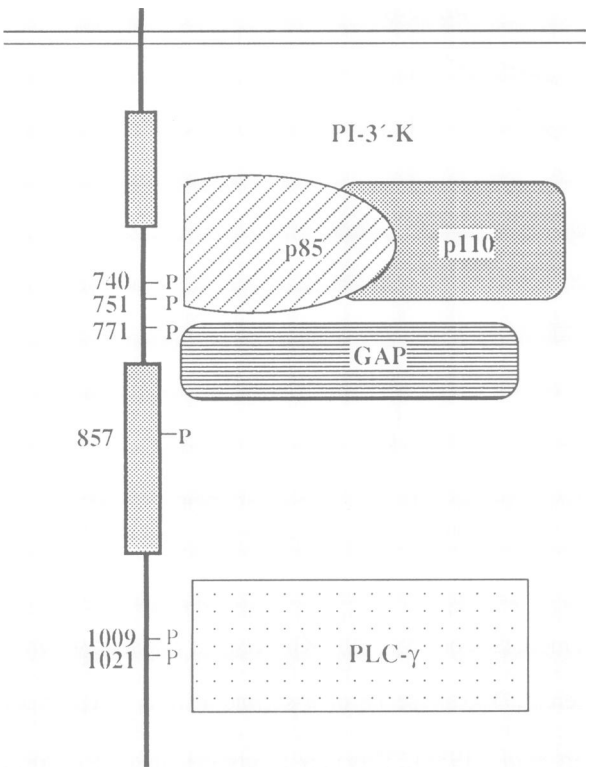

Fig. 3. Schematic illustration of the specificity in the interaction between p85 of phosphatidylinositol-3′-kinase (PI-3′-K), GTPase activating protein (GAP) and phospholipase C-γ (PLC-γ) with defined autophosphorylated tyrosine residues in the cytoplasmic part of the PDGF β-receptor. (Modified from Rönnstrand *et al.*, 1992.)

dimerization. Moreover, ligand-induced receptor dimerization was demonstrated for the EGF receptor (Yarden and Schlessinger, 1987). Using purified preparations of PDGF β-receptors, we directly demonstrated dimerization of the receptor after ligand binding (Heldin,C.-H. *et al.*, 1989). Furthermore, we (Eriksson *et al.*, 1992a; Hammacher *et al.*, 1989) and others (Bishayee *et al.*, 1989; Kanakaraj *et al.*, 1991; Seifert *et al.*, 1989) provided evidence that different types of dimeric complexes were formed by different PDGF isoforms; PDGF-AA caused αα receptor complexes, PDGF-AB αα or αβ receptor complexes and PDGF-BB αα, αβ or ββ receptor complexes (Figure 2).

Why this complexity? This question does not yet have a complete answer, but a detailed comparison of the functional activities of the α- and β-receptors, transfected individually into porcine aortic endothelial cells, revealed similarities as well as differences. Whereas both receptors transduced a mitogenic response, only the β-receptor mediated chemotaxis and actin reorganization in the form of circular membrane ruffles (Eriksson *et al.*, 1992b). In fact, when assayed in cells having both α- and β-receptors, activation of the α-receptor actually inhibited β-receptor-induced chemotaxis (Siegbahn *et al.*, 1990; Vassbotn *et al.*, 1992).

Receptor dimerization leads to autophosphorylation of the receptors, which has been shown to occur *in trans* between the two receptor molecules in the dimer (Kelly *et al.*, 1991). Dimerization might be sufficient for activation of the PDGF receptor, since autophosphorylation was seen also in the absence of ligand after dimerization induced by a monoclonal antibody against the receptor (Rönnstrand *et al.*, 1988). The autophosphorylation appears to serve two purposes; it locks the kinase in an active configuration and provides attachment sites for putative substrate molecules (reviewed by Ullrich and Schlessinger, 1990). An interesting concept has been established over the last years; a conserved motif in the putative substrates, designated Src homology region 2 (SH2), mediates the interaction with phosphorylated sites in the receptors (reviewed in Koch *et al.*, 1991). Studies on the PDGF β-receptor, performed in many different laboratories, have been quite informative; this receptor associates with most of the well characterized SH2-domain containing substrates for protein tyrosine kinase receptors, i.e. phosphatidylinositol-3′-kinase (PI-3-K), GTPase activating protein (GAP), phospholipase C-γ (PLC-γ) and Src (reviewed by Cantley *et al.*, 1991; Ullrich and Schlessinger, 1990).

Many different PDGF receptor mutants have been constructed and characterized, by us and others, with the aim of linking the functional properties of the receptor with specific structural domains. Since the autophosphorylation sites are so important for the interaction with downstream components of the single transduction machinery, the most useful information has come from studies of mutants in which specific autophosphorylation sites were mutated. Currently, six autophosphorylation sites have been mapped in the PDGF β-receptor; Tyr740, Tyr751 and Tyr771 in the kinase insert, Tyr857 in the second part of the kinase domain (Fantl *et al.*, 1992; Kashishian *et al.*, 1992; Kazlauskas and Cooper, 1989), and Tyr1009 and Tyr1021 in the C-terminal tail (Rönnstrand *et al.*, 1992). Interestingly, a specificity in the interaction between the known substrates and the various autophosphorylation sites was unravelled; Tyr740 and Tyr751 mediate the binding of PI-3′-K, and Tyr771 mediates the binding of GAP (Fantl *et al.*, 1992; Kashishian *et al.*, 1992), whereas Tyr1009 and Tyr1021 mediate the binding of PLC-γ (Rönnstrand *et al.*, 1992) (Figure 3). Important goals for future studies are now to determine the role of these and other substrates in the signal pathways leading to e.g. cell growth and chemotaxis.

Control mechanisms

Ligand binding, followed by receptor dimerization and autophosphorylation thus initiates the mitogenic signal, but how is the signal shut off? The fate of the ligand–receptor complex is to be internalized and ultimately degraded in the lysosomes, which usually occurs within one hour. However, we observed that PDGF needs to be present for >8 h in the conditioned medium of human fibroblasts in order to elicit any appreciable mitogenic response (Westermark and Heldin, 1985). It thus appears that there is a need for the formation of novel activated ligand–receptor complexes continuously over an extended time period in order to commit the cell to division. It is thus likely that the average time that the ligand–receptor complexes are in an active configuration is an important factor in the commitment to mitogenicity. One possible shut off mechanism would be the dephosphorylation of autophosphorylation sites of the receptor through the action of protein tyrosine phosphatases, another would be proteolytic degradation in the cytoplasm prior to the fusion of endocytic vesicles with lysosomes. We recently made an observation of potential relevance to the latter possibility; we found that the PDGF β-receptor

undergoes a transient ubiquitination in a ligand-dependent manner (Mori *et al.*, 1992). This finding was not entirely unexpected since Yarden *et al.* (1986) had reported that a preparation of PDGF receptor purified from 3T3 cells contained ubiquitin. Ubiquitin is a 76 amino acid polypeptide which becomes covalently attached to lysine residues or to the N-terminus of certain proteins and thereby marks them for degradation by a proteolytic system in the cytoplasm. Interestingly, the ubiquitination is dependent on Tyr1009 and Tyr1021, the same sites which after autophosphorylation mediate the interaction with PLC-γ (Mori *et al.*, submitted for publication). Consistent with a possible regulatory role of ubiquitination in cell growth, we found that receptor mutants in which both Tyr1009 and Tyr1021 were replaced with phenylalanine residues were more potent in mediating mitogenicity after PDGF stimulation than wild-type receptors (Mori *et al.*, submitted for publication).

Biosynthesis and processing of PDGF

Both polypeptide chains of PDGF are synthesized as precursors with signal sequences; the mature molecules are formed by N-terminal and C-terminal processing. After we realized that the two polypeptide chains of PDGF form different types of dimers, an important question to resolve was whether there is a mechanism in cells to determine which isoforms are assembled. Overexpression of the two chains simultaneously in CHO cells led to the formation of all three isoforms with the heterodimer being the predominant species (Östman *et al.*, 1988), which is the expected result of a random assembly process. The more recent finding that mixing of bacterially synthesized PDGF A- and B-chains gives PDGF-AB as the predominant product (Hoppe *et al.*, 1990), is consistent with the notion that the assembly of the heterodimer does not require any specialized cellular machinery.

We noticed a clear difference in the secretory behaviours of the different PDGF isoforms synthesized by the CHO cells; most PDGF-BB remained associated with the producer cell and was not properly secreted (Östman *et al.*, 1988, 1992). This observation was consistent with previous observations on SSV-transformed cells; the v-*sis* product was found to remain cell-associated and to undergo further proteolytic processing to a 24 kDa form (Robbins *et al.*, 1983, 1985). We subsequently showed that retention occurred in the endoplasmic reticulum and the Golgi complex (Thyberg *et al.*, 1990) and that the retention was dependent on a basic stretch of amino acids in the C-terminal part of the B-chain precursor (Östman *et al.*, 1991). Interestingly, the A-chain occurs in two splice variants, one longer variant in which a similar motif is present in the C-terminus, and a shorter variant without such a sequence (Bonthron *et al.*, 1988; Rorsman *et al.*, 1988). No differences in receptor binding properties of the two forms were observed (Östman *et al.*, 1989). However, the long A-chain was retained like the B-chain, while the short A-chain variant was not (Östman *et al.*, 1991). Thus, the compartmentalization of the A-chain forms is determined by differential splicing. Interestingly, Raines and Ross (1992) recently reported that this sequence can also mediate interaction with heparan sulfate proteoglycans of the connective tissue. These observations on PDGF, together with analogous observations on other growth factors, suggest that differential compartmentalization of growth factors can have potentially important consequences for their functional activities.

PDGF in cancer

The gene for the PDGF B-chain was cloned soon after the demonstration of the homology with *sis* (Johnsson *et al.*, 1984; Josephs *et al.*, 1984); the A-chain gene was cloned somewhat later (Betscholtz *et al.*, 1986a). By DNA transfection it was shown that the normal PDGF B-chain gene has transforming properties (Clarke *et al.*, 1984; Gazit *et al.*, 1984), as does the A-chain gene, albeit with less potency (Beckmann *et al.*, 1988; Bywater *et al.*, 1988). The important question now was whether inappropriate expression of PDGF or PDGF receptors was also of importance in human malignancies. Extensive screening for PDGF production in tumour cell lines, in our own laboratory as well as in other laboratories, revealed that PDGF A- and B-chains are commonly expressed in such cell lines (reviewed in Heldin and Westermark, 1989; Raines *et al.*, 1990). Several of the cell lines investigated showed expression of both A- and B-chains, yet PDGF-AA was always the predominant species secreted (Hammacher *et al.*, 1988b), consistent with the differences in the compartmentalization of the different isoforms of PDGF discussed above.

Using the human osteosarcoma cell line U-2OS, we tried to determine whether the endogenous PDGF production provided the cells with growth advantage. We were able to demonstrate an autocine PDGF receptor activation, but effects on cell growth were more difficult to show, probably because of the numerous genetic alterations the cells had acquired during several years of *in vitro* culturing (Betsholtz *et al.*, 1984). More recent studies have provided several examples of autocrine growth stimulation of tumour cells, involving PDGF and other growth factors (reviewed in Heldin and Westermark, 1989).

Due to Bengt Westermark's long standing interest in human gliomas, we have been particularly interested in the expression of PDGF in human brain tumours. Of 23 different human glioblastoma cell lines examined, 17 were found to express PDGF B-chain and all of them the A-chain (Nistér *et al.*, 1988b). Most of the cell lines also expressed PDGF α- or β-receptors, suggesting that autocrine stimulation occurred (Nistér *et al.*, 1991). Further analysis of the expression of mRNA and protein for PDGF and PDGF receptors in sections of human glioblastomas, provided evidence that the two PDGF receptors are both involved in stimulation of glioblastoma growth, but in different ways; the α-receptor was expressed mainly in the tumour cells whereas the β-receptor was expressed in the supporting stroma (Hermansson *et al.*, 1988, 1992). The PDGF A- and B-chains were also expressed. Importantly, the expression of the PDGF A- and B-chains in the tumour cells and of the β-receptor in the stroma were higher in more malignant tumours than in benign or less malignant tumours, suggesting that the autocrine and paracrine effects of PDGF increase with the degree of malignancy. A similar malignancy-dependent expression of PDGF and PDGF receptors was also seen in fibrosarcomas (Smits *et al.*, 1992).

The studies on glioblastomas suggested that the PDGF β-receptor might be of particular importance for the development of the supporting stromal tissue. This notion

was also supported by studies on human carcinoid tumours in which the β-receptor was found to be expressed at high levels in the stroma (Funa *et al.*, 1990). In order to explore this further, the PDGF B-chain was transfected into human melanoma cells which lack PDGF receptors and thus do not themselves respond to the endogenously produced factor. Comparison of the histology of tumours formed in nude mice of melanoma cells producing and not producing PDGF revealed a striking difference; the tumours of melanoma cells that did not produce PDGF showed central necroses, whereas those that made PDGF contained a well-developed stroma that kept the tumour cells well-nourished and prevented necrosis (Forsberg *et al.*, 1992). Different isoforms of PDGF may thus, through autocrine and paracrine mechanisms, stimulate the balanced growth of different cell types in certain tumours.

PDGF *in vivo*

As discussed above, aberrant expression of PDGF or of PDGF receptors is likely to be involved in the stimulation of the growth of certain tumours. However, over-activity of PDGF could also be part of the development of certain non-malignant disorders involving an excess of cell proliferation. Examples include atherosclerosis, where PDGF-induced stimulation of smooth muscle cell proliferation could contribute to the thickening of the intima of affected vessels (Ross, 1986), as well as chronic fibrotic processes, where PDGF could be involved in the stimulation of connective tissue cell proliferation. In considering the role of PDGF in different diseases, it is important to take into account the fact that PDGF receptor levels can be modulated. For example, we observed that the smooth muscle cells in myometrial tissue contain only very low amounts of PDGF β-receptors, whereas the level of receptors is upregulated in conjunction with *in vitro* culturing of the cells (Terracio *et al.*, 1988). Moreover, we observed increased expression of the PDGF β-receptor in conjunction with various inflammatory conditions (Rubin *et al.*, 1988b), one example being the joints of patients with rheumatoid arthritis (Rubin *et al.*, 1988a).

What then could be the normal function of PDGF? The fact that PDGF and PDGF receptors are expressed in embryonal tissues (Mercola *et al.*, 1990) and in the placenta (Goustin *et al.*, 1985; Holmgren *et al.*, 1992), suggests a function for PDGF during development. In fact, a role for PDGF in the differentiation of glial cells of the rat optic nerve has been proven (Noble *et al.*, 1988; Richardson *et al.*, 1988). Moreover, PDGF (Sasahara *et al.*, 1991; Yeh *et al.*, 1991) and PDGF receptors (Smits *et al.*, 1991) are present in neurons, and PDGF has neurotrophic activity for neuronal cells of rat brain (Smits *et al.*, 1991). PDGF and PDGF receptors are also expressed in the peripheral nervous system (Eccleston *et al.*, 1992). PDGF might thus have a function during the development and maintenance of the central as well as the peripheral nervous system.

The fact that PDGF stimulates growth as well as chemotaxis of connective tissue cells, and also chemotaxis of inflammatory cells (Deuel *et al.*, 1982; Siegbahn *et al.*, 1990), is consistent with a role in wound healing. Moreover, PDGF stimulates the production of matrix constituents, including hyaluronan (Heldin,P. *et al.*, 1989). Recently, the results of the first clinical trial with PDGF were published; a stimulatory effect of high doses of locally applied PDGF-BB on the healing of large bed sores was reported (Robson *et al.*, 1992). The fact that PDGF β-receptors occur on capillary endothelial cells (Bar *et al.*, 1989; Smits *et al.*, 1989) and that PDGF has weak angiogenic activity (Risau *et al.*, 1992), might also be important for its stimulatory effect on wound healing.

Antagonists of PDGF

The adverse effects of PDGF in certain diseases, as discussed above, make PDGF antagonists highly desirable. We and others have recently taken several approaches to develop such antagonists. Antibodies against PDGF have proven to be useful for inhibiting both autocrine stimulation in SSV-transformed cells (Johnsson *et al.*, 1985b) and the atherosclerotic process that occurs after de-endothelialization of the carotid arteries of rats (Ferns *et al.*, 1991). Moreover, a soluble form of the PDGF receptor has been shown to bind and inactivate PDGF (Duan *et al.*, 1991), and could thus be potentially useful for inhibiting PDGF action *in vivo*.

Another approach would be to design or find agents that compete in an antagonistic manner with PDGF for receptor binding. In order to identify peptides that interfere with PDGF binding, we systematically screened peptides derived from the B-chain sequence. One peptide was found that inhibited PDGF binding and autophosphorylation of α- as well as β-receptors (Engström *et al.*, 1992). However, the peptide also showed some cell toxicity and further development will be necessary before peptide antagonists become useful for *in vivo* studies. Low molecular weight compounds that interfere with receptor binding have been described, e.g. suramin (Williams *et al.*, 1984). However, suramin is not specific enough to be clinically useful as a PDGF antagonist (Betsholtz *et al.*, 1986a). We recently found that another low molecular weight compound, neomycin, at high concentrations inhibited the binding of PDGF-BB to the α-receptor, but did not inhibit binding to the β-receptor (Vassbotn *et al.*, 1992). This compound thus represents an antagonist that distinguishes between the two receptor types; however, its low potency makes it unsuitable for use *in vivo*. Hopefully, the experiences with suramin and neomycin will aid the future design of more potent and specific PDGF receptor antagonists. The design of such antagonists would be much facilitated by the elucidation of the three-dimensional structure of the PDGF–receptor complex.

PDGF antagonistic activity could also be achieved by inhibition of PDGF receptor dimerization. We hypothesized that monomeric PDGF might fail to induce receptor dimerization and might thus have antagonistic activity. Since reduction of PDGF results in loss of receptor binding, we attempted to identify the interchain disulfide bonds in order to mutate these residues and thereby prevent dimerization of the ligand. This turned out to be quite difficult due to the high density of cysteine residues in PDGF. The approach that finally succeeded involved partial reduction of the PDGF molecule using a concentration of dithiothreitol that reduced only the interchain disulfide bonds, and left the intrachain bonds unaffected. By this procedure the second and fourth cysteine residues from the N-terminus were found to form the two interchain bonds in PDGF (Andersson *et al.*, 1992). Analysis of a PDGF B-chain mutant in which these two cysteine residues had been mutated to serine residues

revealed that it retained receptor binding activity. Is it a receptor antagonist? The answer is no, in fact, the monomeric PDGF induced both receptor dimerization and autophosphorylation (Andersson *et al.*, 1992). This result may indicate that PDGF-induced receptor dimerization is not only a matter of forming a bridge between two receptor molecules; the dimerization may also involve a ligand-induced conformational change of the extracellular domains of the receptors which promotes receptor–receptor interactions. One possible way of achieving an antagonistic effect, which we are currently exploring, is to combine a wild-type PDGF chain with a mutated chain that does not bind to PDGF receptors but can actively prevent dimerization of receptors.

Concluding remarks

The development of the field of growth factor research since 1975 when I started my thesis project, has been remarkable. It has been truly exciting to follow the expansion in terms of numbers of factors and receptors known, and the increase in knowledge about their structural and functional properties. At present, the field is more interesting than ever. The availability of large quantities of factors through recombinant techniques will now make it possible to determine their three-dimensional structures and to conduct clinical trials in order to explore their possible utility. The availability of the technology to knock out genes will make it possible to obtain an appreciation of the *in vivo* function of growth factors. Finally, the contours of the intracellular machinery for signal transduction are now emerging. I was fortunate to be introduced into this research field, and I have never seriously considered working on anything else.

Acknowledgements

The studies described in this communication would not have been possible without the advice, support and active contributions of a number of collaborators. I would like to express my sincere thanks to all these colleagues; they are too many to be mentioned individually here, but the names of most of them can be found in the cited literature. I would also like to thank the Finnish Red Cross, Helsinki (G.Myllylä) and the Department of Virology, Uppsala (G.Alm) for the supply of large quantities of human platelets which was a prerequisite for the early phase of these studies. I also thank L.Claesson-Welsh and B.Westermark for valuable comments on this review, and I.Schiller for help in its preparation.

References

Andersson,M., Östman,A., Bäckström,G., Hellman,U., George-Nascimento,C., Westermark,B. and Heldin,C.-H. (1992) *J. Biol. Chem.*, **267**, 11260–11266.

Antoniades,H.N. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 7314–7317.

Antoniades,H.N. and Hunkapiller,M.W. (1983) *Science*, **220**, 963–965.

Antoniades,H.N., Scher,C.D. and Stiles,C.D. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 1809–1812.

Bar,R.S., Boes,M., Booth,B.A., Dake,B.L., Henley,S. and Hart,M.N. (1989) *Endocrinology*, **124**, 1841–1848.

Beckmann,M.P., Betsholtz,C., Heldin,C.-H., Westermark,B., Di Marco,E., Di Fiore,P.P., Robbins,K.C. and Aaronson,S.A. (1988) *Science*, **241**, 1346–1349.

Betsholtz,C., Westermark,B., Ek,B. and Heldin,C.-H. (1984) *Cell*, **39**, 447–457.

Betsholtz,C., Johnsson,A., Heldin,C.-H. and Westermark,B. (1986a) *Proc. Natl. Acad. Sci. USA*, **83**, 6440–6444.

Betsholtz,C., Johnsson,A., Heldin,C.-H., Westermark,B., Lind,P., Urdea,M.S., Eddy,R., Shows,T.B., Philpott,K., Mellor,A.L., Knott,T.J. and Scott,J. (1986b) *Nature*, **320**, 695–699.

Bishayee,S., Majumdar,S., Khire,J. and Das,M. (1989) *J. Biol. Chem.*, **264**, 11699–11705.

Bonthron,D.T., Morton,C.C., Orkin,S.H. and Collins,T. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 1492–1496.

Bowen-Pope,D.F. and Ross,R. (1982) *J. Biol. Chem.*, **257**, 5161–5171.

Bywater,M., Rorsman,F., Bongcam-Rudloff,E., Mark,G., Hammacher,A., Heldin,C.-H., Westermark,B. and Betsholtz,C. (1988) *Mol. Cell. Biol.*, **8**, 2753–2762.

Cantley,L.C., Auger,K.R., Carpenter,C., Duckworth,B., Graziani,A., Kapeller,R. and Soltoff,S. (1991) *Cell*, **64**, 281–302.

Carpenter,G. and Cohen,S. (1979) *Annu. Rev. Biochem.*, **48**, 193–216.

Claesson-Welsh,L., Eriksson,A., Morén,A., Severinsson,L., Ek,B., Östman,A., Betsholtz,C. and Heldin,C.-H. (1988) *Mol. Cell. Biol.*, **8**, 3476–3486.

Claesson-Welsh,L., Eriksson,A., Westermark,B. and Heldin,C.-H. (1989a) *Proc. Natl. Acad. Sci. USA*, **86**, 4917–4921.

Claesson-Welsh,L., Hammacher,A., Westermark,B., Heldin,C.-H. and Nistér,M. (1989b) *J. Biol. Chem.*, **264**, 1742–1747.

Clarke,M.F., Westin,E., Schmidt,D., Josephs,S.F., Ratner,L., Wong-Staal,F., Gallo,R.C. and Reitz,M.S.J. (1984) *Nature*, **308**, 464–467.

Deuel,T.F., Huang,J.S., Proffitt,R.T., Baenziger,J.U., Chang,D. and Kennedy,B.B. (1981) *J. Biol. Chem.*, **256**, 8896–8899.

Deuel,T.F., Senior,R.M., Huang,J.S. and Griffin,G.L. (1982) *J. Clin. Invest.*, **69**, 1046–1049.

Devare,S.G., Reddy,E.P., Law,J.D., Robbins,K.C. and Aaronson,S.A. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 731–735.

Doolittle,R.F., Hunkapiller,M.W., Hood,L.E., Devare,S.G., Robbins,K.C., Aaronson,S.A. and Antoniades,H.N. (1983) *Science*, **221**, 275–277.

Duan,D.-S.R., Pazin,M.J., Fretto,L.J. and Williams,L.T. (1991) *J. Biol. Chem.*, **266**, 413–418.

Eccleston,P.A., Funa,K. and Heldin,C.-H. (1992) *Dev. Biol.*, in press.

Ek,B. and Heldin,C.-H. (1982) *J. Biol. Chem.*, **257**, 10486–10492.

Ek,B. and Heldin,C.-H. (1984) *J. Biol. Chem.*, **259**, 11145–11152.

Ek,B., Westermark,B., Wasteson,Å. and Heldin,C.-H. (1982) *Nature*, **295**, 419–420.

Engström,U., Engström,Å., Ernlund,A., Westermark,B. and Heldin,C.-H. (1992) *J. Biol. Chem.*, **267**, 16581–16587.

Eriksson,A., Rorsman,C., Ernlund,A., Claesson-Welsh,L. and Heldin,C.-H. (1992a) *Growth Factors*, **6**, 1–14.

Eriksson,A., Siegbahn,A., Westermark,B., Heldin,C.-H. and Claesson-Welsh,L. (1992b) *EMBO J.*, **11**, 543–550.

Escobedo,J.A., Barr,P.J. and Williams,L.T. (1988) *Mol. Cell. Biol.*, **8**, 5126–5131.

Fantl,W.J., Escobedo,J.A., Martin,G.A., Turck,C.W., del Rosario,M., McCormick,F. and Williams,L.T. (1992) *Cell*, **69**, 413–423.

Ferns,G.A.A., Raines,E.W., Sprugel,K.H., Motani,A.S., Reidy,M.A. and Ross,R. (1991) *Science*, **253**, 1129–1132.

Forsberg,K., Valyi-Nagi,I., Heldin,C.-H., Herlyn,M. and Westermark,B. (1992) *Proc. Natl. Acad. Sci. USA*, in press.

Funa,K., Papanicolaou,V., Juhlin,C., Rastad,J., Åkerström,G., Heldin,C.-H. and Öberg,K. (1990) *Cancer Res.*, **50**, 748–753.

Gazit,A., Igarashi,H., Chiu,I.-M., Srinivasan,A., Yaniv,A., Tronick,S.R., Robbins,K.C. and Aaronson,S.A. (1984) *Cell*, **39**, 89–97.

Glenn,K., Bowen-Pope,D.F. and Ross,R. (1982) *J. Biol. Chem.*, **257**, 5172–5176.

Goustin,A.S., Betsholtz,C., Pfeifer-Ohlsson,S., Persson,H., Rydnert,J., Bywater,M., Holmgren,G., Heldin,C.-H., Westermark,B. and Ohlsson,R. (1985) *Cell*, **41**, 301–312.

Hammacher,A., Hellman,U., Johnsson,A., Östman,A., Gunnarson,K., Westermark,B., Wasteson,Å. and Heldin,C.-H. (1988a) *J. Biol. Chem.*, **263**, 16493–16498.

Hammacher,A., Nistér,M., Westermark,B. and Heldin,C.-H. (1988b) *Eur. J. Biochem.*, **176**, 179–186.

Hammacher,A., Mellström,K., Heldin,C.-H. and Westermark,B. (1989) *EMBO J.*, **8**, 2489–2495.

Hart,C.E., Forstrom,J.W., Kelly,J.D., Seifert,R.A., Smith,R.A., Ross,R., Murray,M.J. and Bowen-Pope,D.F. (1988) *Science*, **240**, 1529–1531.

Hart,C.E., Bailey,M., Curtis,D.A., Osborn,S., Raines,E., Ross,R. and Forstrom,J.W. (1990) *Biochemistry*, **29**, 166–172.

Heldin,C.-H. and Westermark,B. (1984) *Cell*, **37**, 9–20.

Heldin,C.-H. and Westermark,B. (1989) *Eur. J. Biochem.*, **184**, 487–496.

Heldin,C.-H. and Westermark,B. (1990) *Cell Regulation*, **1**, 555–566.

Heldin,C.-H., Westermark,B. and Wasteson,Å. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3722–3726.

Heldin,C.-H., Westermark,B. and Wasteson,Å. (1980) *J. Cell. Physiol.*, **105**, 235–246.

Heldin,C.-H., Westermark,B. and Wasteson,Å. (1981a) *Biochem. J.*, **193**, 907–913.
Heldin,C.-H., Westermark,B. and Wasteson,Å. (1981b) *Proc. Natl. Acad. Sci. USA*, **78**, 3664–3668.
Heldin,C.-H., Wasteson,Å. and Westermark,B. (1982) *J. Biol. Chem.*, **257**, 4216–4221.
Heldin,C.-H., Ek,B. and Rönnstrand,L. (1983) *J. Biol. Chem.*, **258**, 10054–10061.
Heldin,C.H., Johnsson,A., Wennergren,S., Wernstedt,C., Betsholtz,C. and Westermark,B. (1986) *Nature*, **319**, 511–514.
Heldin,C.-H., Johnsson,A., Ek,B., Wennergren,S., Rönnstrand,L., Hammacher,A., Faulders,B., Wasteson,Å. and Westermark,B. (1987) *Methods Enzymol.*, **147**, 3–13.
Heldin,C.-H., Bäckström,G., Östman,A., Hammacher,A., Rönnstrand,L., Rubin,K., Nistér,M. and Westermark,B. (1988) *EMBO J.*, **7**, 1387–1393.
Heldin,C.-H., Ernlund,A., Rorsman,C. and Rönnstrand,L. (1989) *J. Biol. Chem.*, **264**, 8905–8912.
Heldin,P., Laurent,T.C. and Heldin,C.-H. (1989) *Biochem. J.*, **258**, 919–922.
Hermansson,M., Nistér,M., Betsholtz,C., Heldin,C.-H., Westermark,B. and Funa,K. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 7748–7752.
Hermansson,M., Funa,K., Hartman,M., Claesson-Welsh,L., Heldin,C.-H., Westermark,B. and Nistér,M. (1992) *Cancer Res.*, **52**, 3213–3219.
Holmgren,L., Claesson,-Welsh,L., Heldin,C.-H., and Ohlsson,R. (1992) *Growth Factors*, **6**, 219–232.
Hoppe,J., Weich,H.A., Eichner,W. and Tatje,D. (1990) *Eur. J. Biochem.*, **187**, 207–214.
Huang,J.S., Huang,S.S., Kennedy,B. and Deuel,T.F. (1982) *J. Biol. Chem.*, **257**, 8130–8136.
Ishikawa,F., Miyazono,K., Hellman,U., Drexler,H., Wernstedt,C., Hagiwara,K., Usuki,K., Takaku,F., Risau,W. and Heldin,C.-H. (1989) *Nature*, **338**, 557–562.
Johnsson,A., Heldin,C.-H., Westermark,B. and Wasteson,Å. (1982) *Biochem. Biophys. Res. Commun.*, **104**, 66–74.
Johnsson,A., Heldin,C.-H., Wasteson,Å., Westermark,B., Deuel,T.F., Huang,J.S., Seeburg,P.H., Gray,A., Ullrich,A., Scrace,G., Stroobant,P. and Waterfield,M.D. (1984) *EMBO J.*, **3**, 921–928.
Johnsson,A., Betsholtz,C., Heldin,C.-H. and Westermark,B. (1985a) *Nature*, **317**, 438–440.
Johnsson,A., Betsholtz,C., von der Helm,K., Heldin,C-H. and Westermark,B. (1985b) *Proc. Natl. Acad. Sci. USA*, **82**, 1721–1725.
Johnsson,A., Betsholtz,C., Heldin,C.-H. and Westermark,B. (1986) *EMBO J.*, **5**, 1535–1541.
Josephs,S.F., Guo,C., Ratner,L. and Wong-Staal,F. (1984) *Science*, **223**, 487–491.
Kanakaraj,P., Raj,S., Khan,S.A. and Bishayee,S. (1991) *Biochemistry*, **30**, 1761–1767.
Kashishian,A., Kazlauskas,A. and Cooper,J.A. (1992) *EMBO J.*, **11**, 1373–1382.
Kazlauskas,A. and Cooper,J.A. (1989) *Cell*, **58**, 1121–1133.
Kelly,J.D., Haldeman,B.A., Grant,F.J., Murray,M.J., Seifert,R.A., Bowen-Pope,D.F., Cooper,J.A. and Kazlauskas,A. (1991) *J. Biol. Chem.*, **266**, 8987–8992.
Koch,C.A., Anderson,D., Moran,M.F., Ellis,C. and Pawson,T. (1991) *Science*, **252**, 668–674.
Kohler,N. and Lipton,A. (1974) *Exp. Cell Res.*, **87**, 297–301.
Matsui,T., Heidaran,M., Miki,T., Toru,M., Popescu,N., La Rochelle,W., Kraus,M., Pierce,J. and Aaronson,S.A. (1989) *Science*, **243**, 800–803.
Mercola,M., Wang,C., Kelly,J., Brownlee,C., Jackson-Grusby,L., Stiles,C. and Bowen-Pope,D. (1990) *Dev. Biol.*, **138**, 114–122.
Miyazono,K., Okabe,T., Urabe,A., Takaku,F. and Heldin,C.-H. (1987) *J. Biol. Chem.*, **262**, 4098–4103.
Miyazono,K., Hellman,U., Wernstedt,C. and Heldin,C.-H. (1988) *J. Biol. Chem.*, **263**, 6407–6415.
Mori,S., Heldin,C.-H. and Claesson-Welsh,L. (1992) *J. Biol. Chem.*, **267**, 6429–6434.
Nistér,M., Hammacher,A., Mellström,K., Siegbahn,A., Rönnstrand,L., Westermark,B. and Heldin,C.-H. (1988a) *Cell*, **52**, 791–799.
Nistér,M., Libermann,T.A., Betsholtz,C., Pettersson,M., Claesson-Welsh,L., Heldin,C.-H., Schlessinger,J. and Westermark,B. (1988b) *Cancer Res.*, **48**, 3910–3918.
Nistér,M., Claesson-Welsh,L., Eriksson,A., Heldin,C.-H. and Westermark,B. (1991) *J. Biol. Chem.*, **266**, 16755–16763.
Noble,M., Murray,K., Stroobant,P., Waterfield,M.D. and Riddle,P. (1988) *Nature*, **333**, 560–562.
Östman,A., Rall,L., Hammacher,A., Wormstead,M.A., Coit,D., Valenzuela,P., Betsholtz,C., Westermark,B. and Heldin,C.-H. (1988) *J. Biol. Chem.*, **263**, 16202–16208.
Östman,A., Bäckström,G., Fong,N., Betsholtz,C., Wernstedt,C., Hellman,U., Westermark,B., Valenzuela,P. and Heldin,C.-H. (1989) *Growth Factors*, **1**, 271–281.
Östman,A., Andersson,M., Betsholtz,C., Westermark,B. and Heldin,C.-H. (1991) *Cell Regul.*, **2**, 503–512.
Östman,A., Thyberg,J., Westermark,B. and Heldin,C.-H. (1992) *J. Cell. Biol.*, **118**, 509–519.
Raines,E.W. and Ross,R. (1982) *J. Biol. Chem.*, **257**, 5154–5160.
Raines,E.W. and Ross,R. (1992) *J. Cell Biol.*, **116**, 533–543.
Raines,E.W., Bowen-Pope,D.F. and Ross,R. (1990) In Sporn,M.B. and Roberts,A.B. (eds), *Handbook of Experimental Pharmacology. Peptide growth factors and their receptors.* Springer-Verlag, Heidelberg, Vol. 95, Part I, pp. 173–262.
Richardson,W.D., Pringle,N., Mosley,M.J., Westermark,B. and Dubois-Dalcq,M. (1988) *Cell*, **53**, 309–319.
Risau,W., Drexler,H., Mironov,V., Smits,A., Siegbahn,A., Funa,K. and Heldin,C.-H. (1992) *Growth Factors*, in press.
Robbins,K.C., Antoniades,H.N., Devare,S.G., Hunkapiller,M.W. and Aaronson,S.A. (1983) *Nature*, **305**, 605–609.
Robbins,K.C., Leal,F., Pierce,J.H. and Aaronson,S.A. (1985) *EMBO J.*, **4**, 1783–1792.
Robson,M.C., Phillips,L.G., Thomason,A., Robson,L.E. and Pierce,G.F. (1992) *Lancet*, **339**, 23–25.
Rönnstrand,L., Beckmann,M.P., Faulders,B., Östman,A., Ek,B. and Heldin,C.-H. (1987) *J. Biol. Chem.*, **262**, 2929–2932.
Rönnstrand,L., Terracio,L., Claesson-Welsh,L., Heldin,C.-H. and Rubin,K. (1988) *J. Biol. Chem.*, **263**, 10429–10435.
Rönnstrand,L., Mori,S., Jonsson,A.-K., Eriksson,A., Wernstedt,C., Hellman,U., Claesson-Welsh,L. and Heldin,C.-H. (1992) *EMBO J.*, **11**, 3911–3919.
Rorsman,F., Bywater,M., Knott,R.J., Scott,J. and Betsholtz,C. (1988) *Mol. Cell. Biol.*, **8**, 571–577.
Ross,R. (1986) *N. Engl. J. Med.*, **314**, 488–500.
Ross,R., Glomset,J., Kariya,B. and Harker,L. (1974) *Proc. Natl. Acad. Sci. USA*, **71**, 1207–1210.
Rubin,K., Terracio,L., Rönnstrand,L., Heldin,C.-H. and Klareskog,L. (1988a) *Scand. J. Immunol.*, **27**, 285–294.
Rubin,K., Tingström,A., Hansson,G.K., Larsson,E., Rönnstrand,L., Klareskog,L., Claesson-Welsh,L., Heldin,C.-H., Fellström,B. and Terracio,L. (1988b) *Lancet*, **1**, 1353–1356.
Sasahara,M., Fries,J.W.U., Raines,E.W., Gown,A.M., Westrum,L.E., Frosch,M.P., Bonthron,D.T., Ross,R. and Collins,T. (1991) *Cell*, **64**, 217–227.
Seifert,R.A., Hart,C.E., Philips,P.E., Forstrom,J.W., Ross,R., Murray,M. and Bowen-Pope,D.F. (1989) *J. Biol. Chem.*, **264**, 8771–8778.
Siegbahn,A., Hammacher,A., Westermark,B. and Heldin,C.-H. (1990) *J. Clin. Invest.*, **85**, 916–920.
Singh,J.P., Chaikin,M.A. and Stiles,C.D. (1982) *J. Cell Biol.*, **95**, 667–671.
Smits,A., Hermansson,M., Nistér,M., Karnushina,I., Heldin,,C.-H., Westermark,B. and Funa,K. (1989) *Growth Factors*, **2**, 1–8.
Smits,A., Kato,M., Westermark,B., Nistér,M., Heldin,C.-H. and Funa,K. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 8159–8163.
Smits,A., Funa,K., Vassbotn,F.S., Beausang-Linder,M., af Ekenstam,F., Heldin,C.-H., Westermark,B. and Nistér,M. (1992) *Am. J. Pathol.*, **140**, 639–648.
Sorkin,A., Westermark,B., Heldin,C.-H. and Claesson-Welsh,L. (1991) *J. Cell Biol.*, **112**, 469–478.
Sporn,M.B. and Todaro,G.J. (1980) *N. Engl. J. Med.*, **303**, 878–880.
Terracio,L., Rönnstrand,L., Tingström,A., Rubin,K., Claesson-Welsh,L., Funa,K. and Heldin,C.-H. (1988) *J. Cell Biol.*, **107**, 1947–1957.
Thyberg,J., Östman,A., Bäckström,G., Westermark,B. and Heldin,C.-H. (1990) *J. Cell Sci.*, **97**, 219–229.
Ullrich,A. and Schlessinger,J. (1990) *Cell*, **61**, 203–212.
Ushiro,H. and Cohen,S. (1980) *J. Biol. Chem.*, **255**, 8363–8365.
Usuki,K., Saras,J., Waltenberger,J., Miyazono,K., Pierce,G., Thomason,A. and Heldin,C.-H. (1992) *Biochem. Biophys. Res. Commun.*, **184**, 1311–1316.
Vassbotn,F.S., Östman,A., Siegbahn,A., Holmsen,H. and Heldin,C.-H. (1992) *J. Biol. Chem.*, **267**, 15635–15641.
Waterfield,M.D., Scrace,G.T., Whittle,N., Stroobant,P., Johnsson,A., Wasteson,Å., Westermark,B., Heldin,C.-H., Huang,J.S. and Deuel,T.F. (1983) *Nature*, **304**, 35–39.
Westermark,B. and Heldin,C.-H. (1985) *J. Cell. Phys.*, **124**, 43–48.
Westermark,B. and Wasteson,Å. (1975) In Luft,R. and Hall,K. (eds),

Advances in Metabolic Disorders. Academic Press, New York, Vol. 8, pp. 85–100.
Westermark,B. and Wasteson,Å. (1976) *Exp. Cell Res.*, **98**, 170–174.
Westermark,B., Betsholtz,C., Johnsson,A. and Heldin,C.-H. (1987) In Kjeldgaard,N.O. and Forchhammer,J. (eds), *Viral Carcinogenesis*. Munksgaard, Copenhagen, Vol. Alfred Benzon Symposium 24, pp. 445–457.
Westermark,B., Siegbahn,A., Heldin,C.-H. and Claesson-Welsh,L. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 128–132.
Williams,L.T., Tremble,P. and Antoniades,H.N. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 5867–5870.
Williams,L.T., Tremble,P.M., Lavin,M.F. and Sunday,M.E. (1984) *J. Biol. Chem.*, **259**, 5287–5294.
Yarden,Y. and Schlessinger,J. (1987) *Biochemistry*, **26**, 1443–1451.
Yarden,Y., Escobedo,J.A., Kuang,W.-J., Yang-Feng,T.L., Daniel,T.O., Tremble,P.M., Chen,E.Y., Ando,M.E., Harkins,R.N., Francke,Y., Friend,V.A., Ullrich,A. and Williams,L.T. (1986) *Nature*, **323**, 226–232.
Yeh,H.-J., Ruit,K.G., Wang,Y.-X., Parks,W.C., Snider,W.D. and Deuel,T.F. (1991) *Cell*, **64**, 209–216.