Spontaneous dissociation–association of monomers of the human-stem-cell-factor dimer

Hsieng S. LU,* Wen-Chang CHANG, Elizabeth A. MENDIAZ, Michael B. MANN, Keith E. LANGLEY and Yueh-Rong HSU

Amgen Inc., Amgen Center, Thousand Oaks, CA 91320, U.S.A.

In its native state, recombinant human-stem-cell-factor (SCF) dimer can spontaneously and rapidly undergo hybridization when two different SCF dimer species are incubated together. SCF species differing in molecular charge, e.g., a wild-type SCF form and a variant with Asp at position 10 instead of Asn, were used in the hybridization studies; the original species and newly formed dimer hybrid can be separated and quantified by cationic-exchange h.p.l.c. The hybridization reaches an equilibrium where the ratio of hybrid dimer to each of the original species is 2. Kinetic studies of the initial rate of hybridization enable a rate constant for monomer dissociation to be determined. This rate

constant is influenced by pH, temperature and salt concentration. The pH and salt effects suggest that salt bridges between charged amino acids at the monomer–monomer interface may be present. From the temperature effects, the activation energy for monomer dissociation was determined to be 85.6 kJ/mol, which is typical for oligomeric proteins. Heavily glycosylated recombinant SCF from Chinese-hamster ovary cells exchanged equally well with the bacterially derived non-glycosylated SCF, indicating that the attached carbohydrate moieties had no effect on monomer exchange.

INTRODUCTION

Stem-cell factor (SCF), also termed ‘kit ligand’ or ‘mast-cell growth factor’ [1–6], exhibits profound biological function in the early stages of haematopoiesis, and is also involved in the development and function of other cell lineages, including melanocytes and germ cells [7,8]. In the mouse system it is encoded by the steel (*S*) locus, and its receptor, the kit tyrosine kinase, is encoded by the dominant white spotting locus (*W*) [3–6,9,10]. On haematopoietic progenitors *in vitro*, SCF is synergistic with other cytokines and colony-stimulating factors (CSFs) in promoting colony formation [1,2,11–16]. *In vivo*, exogenously provided SCF can increase the levels of progenitors and mature cells of multiple haematopoietic lineages in the bone marrow and peripheral blood [3,17,18]. The mobilization of progenitors to the peripheral blood is striking, and the effects of SCF are synergistic with those of granulocyte CSF [19,20].

SCF is initially synthesized as membrane-associated forms of 248 or 220 amino acids, depending on alternative splicing of exon 6 at the mRNA level. The amino acid sequence encoded by exon 6 contains a proteolytic cleavage site such that a soluble form, of 165 amino acids (SCF^{1–165}), tends to be released from the extracellular domain of the membrane-bound 248-amino-acid SCF, while the 220-amino-acid SCF lacks the cleavage site and tends to remain membrane-bound [21–23]. The naturally occurring soluble SCF is glycosylated at both N-linked and O-linked sites [24,25].

The kit receptor belongs to the type III tyrosine kinase family whose members include receptors for macrophage colony-stimulating factor (M-CSF) and platelet-derived growth factor (PDGF) [26–28]. SCF, M-CSF and PDGF are all dimeric ligands [29,31–33] that mediate receptor dimerization. We have previously described the isolation and characterization of soluble

human SCF^{1–165} recombinantly expressed in *Escherichia coli* in non-glycosylated form, and by Chinese-hamster ovary (CHO) cells in glycosylated form [23,29,30]. These SCFs are fully native and biologically functional. In contrast with the M-CSF and PDGF dimers, whose monomers are disulphide-linked [31–33], both the glycosylated and non-glycosylated SCF dimers contain non-covalently linked monomers, as clearly shown by biochemical and physical chemical analyses under the conditions studied [24,29]. In the present study we make use of wild-type and variant forms of recombinant soluble human SCF^{1–165} to demonstrate that the dimers can undergo rapid monomer interchange with other dimers. We show that cation-exchange h.p.l.c. can readily be employed to detect hybrid dimers and to estimate the rate of monomer interchange in kinetic studies. The rate of hybridization is influenced by temperature, pH, ionic strength and SCF concentration, and is not influenced by attached carbohydrate moieties. This unusual dimer hybridization has rarely been reported for other oligomeric proteins.

MATERIALS AND METHODS

Expression and preparation of recombinant SCFs

The procedures for the expression, fermentation and purification of *E. coli*-derived recombinant human SCF^{1–165} have been described previously [29,30]; the initiating N-terminal methionine residue (numbered as –1) is retained. A variant form with Asp at position 10 instead of Asn (N10D, using the one-letter amino acid notation) was prepared by site-directed mutagenesis as described by Langley et al. [34]. Preparation of recombinant human SCF^{1–165} derived from CHO cells has also been described [23,30]; this material has multiple sites of both N-linked and O-linked sugar attachment.

Abbreviations used: SCF, stem-cell factor; M-CSF, macrophage colony stimulating factor; PDGF, platelet-derived growth factor; CHO, Chinese-hamster ovary.

* To whom correspondence should be addressed.

Detection and quantification of SCF dimer forms

A cation-exchange h.p.l.c. system was used to separate different SCF species, e.g., homodimers of the wild-type and N10D SCFs, and the hybrid heterodimers formed therefrom. SCF samples were diluted into h.p.l.c. buffer A (20 mM sodium acetate, pH 4.5), injected on to a sulphopropyl-resin-based column (TSK 5PW; 4.6 mm × 75 mm) and eluted by a linear gradient from 100 % buffer A to 24 % buffer B (20 mM sodium acetate, pH 4.5, containing 500 mM Na₂SO₄) at a flow rate of 1 ml/min for 30 min. Elution using a steeper gradient could also be carried out in 15 min with no change in results obtained. The h.p.l.c. system (Hewlett-Packard HP1050Ti) had titanium pumps and a multiple-wavelength detector. Elution was monitored at 230 nm and 280 nm. The chromatographic data were analysed by Chem-Station software (Hewlett-Packard) with a PC-Window computer system.

Structural characterization and other methods

Automated N-terminal-amino-acid-sequence analysis was performed as described in [24]. Asp-N endoproteinase digestion of SCFs with reverse-phase h.p.l.c. separation of resulting peptides was also carried out as described [30]. Concentrations of *E. coli*-derived SCFs and CHO-cell-derived SCF were determined by absorbance at 280 nm using absorption coefficients of 0.62 ml/mg·cm respectively. Narrow-range (pH 4.5–6.0) isoelectric-focusing gel electrophoresis was performed as described previously [35].

Kinetic studies of SCF monomer exchange

Equal amounts of wild-type and N10D variant SCFs were mixed so as to give desired buffer conditions and SCF concentration. Incubations were carried out at various temperatures, as presented in the Results section. At the end of any given incubation time, samples were immediately analysed by cation-exchange h.p.l.c. Heterodimer formation was quantified from peak areas at each time point; a small heterodimer area value observed for zero time was subtracted from the values for later time points.

Derivation of kinetic parameters

The following derivation leads to an equation for initial rate (v_0) of hybrid formation after mixing equimolar amounts of two homodimers, such that a dissociation rate constant for monomer dissociation can be arrived at using experimentally measurable parameters. Eqns. (1)–(3) describe monomer–monomer dissociation and association for wild-type, N10D, variant and hybrid SCF in a sample mixture, where A and B are the monomeric SCF for wild-type and N10D mutant, respectively:

k_1 , k_2 , and k_3 represent the association rate constants for the corresponding association reactions, and k_{-1} , k_{-2} , and k_{-3} represent the respective dissociation rate constants. We assume that molecules of A and B associate equally well with each other as with themselves, i.e., $k_1 = k_2 = k_3$ and $k_{-1} = k_{-2} = k_{-3}$.

The rate equation for formation of A is:

$$\frac{d[A]}{dt} = k_{-1}([AA] - [A]) + k_{-3}[AB]$$

Assuming $[AB] = 0$ initially upon mixing, and that $[A] \ll [AA]$, we have:

$$\frac{d[A]}{dt} = k_{-1}[AA]$$

The rate equation for removal of A is:

$$-\frac{d[A]}{dt} = k_{-1}[A]^2 + k_3[A][B]$$

At equilibrium:

$$\frac{d[A]}{dt} = -\frac{d[A]}{dt}$$

$$k_{-1}[AA] = k_1[A]^2 + k_3[A][B] \quad (4)$$

Also, at all times:

$$[AA] = [BB] \text{ and } [A] = [B]$$

so that eqn. (4) becomes:

$$\begin{aligned} k_{-1}[AA] &= k_1[A]^2 + k_3[A]^2 \\ &= 2k_1[A]^2 \text{ (since } k_1 = k_3) \end{aligned}$$

and

$$[A]^2 = \frac{k_{-1}}{2k_1}[AA] \quad (5)$$

The initial velocity for hybrid formation (v_0) is:

$$\begin{aligned} v_0 &= k_3[A][B] \\ &= k_3[A]^2 = k_1[A]^2 \text{ (since } [A] = [B] \text{ and } k_1 = k_3) \\ &= k_1 \frac{k_{-1}[AA]}{2k_1} \text{ (using eqn. 5)} \\ &= \frac{k_{-1}}{2}[AA] \end{aligned} \quad (6)$$

Thus the dissociation rate constant for the dimerization reaction can be obtained as twice the slope of v_0 plotted against $[AA]$.

RESULTS

Detection and characterization of hybrid SCF dimer

When analysed by cation-exchange h.p.l.c., the wild-type and variant *E. coli*-derived SCFs each gave single, but differently eluting, peaks. As shown in Figure 1, the wild-type SCF was eluted at about 28 min (Figure 1a), while the N10D variant was eluted at about 23 min (Figure 1b), reflecting its increased negative charge. This mobility difference provided the basis for detecting hybrid dimers. When the wild-type and N10D variant SCFs were mixed and incubated for 20 h, the intermediate peak apparent in Figure 1(c) was present, representing 50 % of the total peak integration area. In control 20 h incubations of the two forms alone, each retained its behaviour as a single peak on cation-exchange h.p.l.c. with unchanged elution position. The

Figure 1 Cationic-exchange h.p.l.c. of bacterially derived human SCF

(a) Wild-type SCF; (b) N10D SCF variant; (c) after 20 h incubation of wild-type and N10D SCF mixture; incubation was in 10 mM sodium acetate buffer, pH 4.5, at 37 °C with the concentration of wild-type and N10D variant each at 1 mg/ml.

Figure 2 Dimer hybridization between wild-type and N10D SCFs at early incubation times, detected by cationic-exchange h.p.l.c.

(a)–(d) show detection at 0, 5, 7.5, and 10 min after the two SCF forms were mixed. For experimental conditions, see the legend to Figure 1(c).

elution position and relative amount of the intermediate peak in Figure 1(c) clearly indicate that it could represent a hybrid dimer. This was confirmed by several analyses of the intermediate peak material recovered from an h.p.l.c. run. By isoelectric-focusing gel electrophoresis, the material focused with a pI of 4.75, intermediate between that for wild-type SCF (pI 5.0) and the N10D variant SCF (pI 4.7) (results not shown). By N-terminal-amino-acid-sequence analysis, wild-type SCF had only Asn at position 10, and the N10D variant had only Asp, while the intermediate peak material had equal signals for Asn and Asp at this position. The intermediate peak material was also subjected to Asp-N endoproteinase digestion (followed by dithiothreitol treatment to cleave disulphide bonds) with reverse-phase h.p.l.c. separation of the resulting peptides. Asp-N endoproteinase cleaves peptide bonds on the N-terminal side of Asp residues.

Figure 3 Kinetic studies of SCF heterodimer formation

Buffer was 10 mM sodium acetate, pH 4.5, and the temperature was 25 °C. (a) Time course for attainment of equilibrium, with wild-type and N10D variant SCF each at 1.0 mg/ml. (b) Initial rates for different SCF concentrations (squares, SCFs at 1 mg/ml; diamonds, SCFs at 0.5 mg/ml; circles, SCFs at 0.25 mg/ml). (c) Initial rate as a function of SCF concentration; the S.D. of v_0 values was less than 5%, and the correlation coefficient for the plots was greater than 0.98.

The h.p.l.c. profile was typical [30], except that the wild-type N-terminal peptide, M-E-G-I-C-R-N-R-V-T-N-N-V-K, was decreased in amount relative to other peptides, and two unique peptides, M-E-G-I-C-R-N-R-V-T and D-N-V-K, were detected. The isoelectric-focusing and sequencing data clearly demonstrate that the intermediate peak represents SCF heterodimer comprised of a wild-type SCF subunit and an N10D variant subunit. The isolated heterodimer, upon subsequent incubation (e.g. 24 h at

Figure 4 Arrhenius plot to obtain activation energy for SCF monomer dissociation

25 °C), redistributes to the 1:2:1 ratio of wild-type SCF, heterodimer and N10D variant as separated by the cation-exchange h.p.l.c.

Kinetic studies of heterodimer formation

To obtain kinetic data, heterodimer formation was followed as a function of time. Initially, the wild-type and N10D variant (each at 1 mg/ml) were incubated together at 37 °C in 20 mM sodium acetate buffer, pH 4.5. Figures 2(a)–2(d) represent cation-exchange h.p.l.c. of SCF mixtures analysed after 0, 5, 7.5 and 10 min incubation respectively. These data indicate that the hybridization between the wild-type and N10D variant occurs at a fairly rapid rate.

Figure 3(a) shows the time course of hybridization during incubation up to 25 h at 25 °C with wild-type and N10D variant each at 1 mg/ml. Heterodimer formed quickly during the first 5 h and subsequently reached equilibrium, with wild-type SCF, heterodimer and N10D variant at a ratio of 1:2:1. As shown in Figure 3(b), the rate of heterodimer formation is linear over the first 10 min; we therefore calculated initial rate (v_0) over this 10 min period and, as seen in Figure 3(b), and plotted in Figure 3(c), v_0 is dependent on SCF concentration. According to the kinetic equations (see the Materials and methods section), the initial rate constant (k_{-1}) for SCF monomer dissociation can be obtained from the plot of initial rate of heterodimer formation (v_0) versus initial concentration of wild-type or N10D variant (Figure 3c), and was determined to be $1.35 \times 10^{-4} \text{ s}^{-1}$ under these conditions.

The effect of temperature on heterodimer formation was also investigated. The dissociation rate constants obtained at different temperatures (15, 25, 30 and 37 °C) were plotted according to the Arrhenius equation:

$$\ln k_{-1} = -E_a/RT + \ln A$$

where E_a is the activation energy for the dissociation of SCF monomers, R the gas constant, T , the temperature in K, and A an experimental constant. The Arrhenius plot obtained is shown in Figure 4. The activation energy for dissociation of SCF monomers at pH 4.5 is 85.6 kJ/mol.

Effects of pH, salt and SCF glycoforms

The effect of pH on the rate of SCF dimer hybridization is summarized in Table 1. At pH 4.5 and 37 °C formation of SCF heterodimer has an initial rate of $1.35 \times 10^{-2} \text{ mg/min per ml}$ ($k_{-1} = 9 \times 10^{-4} \text{ s}^{-1}$) in sodium acetate buffer with wild type SCF and N10D variant each at 0.5 mg/ml. The initial rate was higher

Table 1 Effect of pH and salt on the rate of SCF dimer hybridization

Conditions*	Initial rate of heterodimer formation (v_0 [mg/min per ml])	k_{-1} (s^{-1})†
pH 3.2	2.56×10^{-2}	1.67×10^{-3}
pH 4.5	1.35×10^{-2}	9.0×10^{-4}
pH 8.2	8.0×10^{-3}	5.33×10^{-5}
pH 4.5, 1 M NaCl	1.69×10^{-2}	1.13×10^{-3}

* The reaction was carried out at 37 °C, using an initial SCF concentration of 0.5 mg/ml for both wild-type SCF and the N10D analogue.

† k_{-1} was obtained from eqn. (6) (see the Materials and methods section), i.e., $k_{-1} = 2v_0/[AA]$.

by about 2-fold at pH 3.2 ($k_{-1} = 1.67 \times 10^{-3} \text{ s}^{-1}$), and lower at pH 8.2 ($k_{-1} = 5.33 \times 10^{-5} \text{ s}^{-1}$). In addition, heterodimer formation was studied in the presence of 1 M NaCl, and the initial rate was found to be approx. 25% higher than that in the absence of salt (Table 1).

We also investigated monomer exchange between wild-type non-glycosylated (*E. coli*-derived) and glycosylated (CHO-cell-derived) SCF dimers. In our cation-exchange h.p.l.c. method, glycosylated SCF dimer is eluted at approx. 3.5–4 min, while the heterodimer is eluted at approx. 10 min. Incubations were initiated with each SCF type at 0.5 mg/ml in buffer, pH 4.5, at 37 °C. The results indicated that the rate of heterodimer formation between the non-glycosylated and glycosylated SCFs is similar to that observed for the two different non-glycosylated species ($v_0 = 1.11 \times 10^{-2} \text{ mg/min per ml}$ and $k_{-1} = 7.4 \times 10^{-4} \text{ s}^{-1}$).

DISCUSSION

In the concentration range of 0.25–1.0 mg/ml, as used in the present studies, SCF is a non-covalently-held homodimer; by sedimentation-equilibrium ultracentrifugation, free monomer is not present, within the limits of detection [29]. The equilibrium constant for dimer formation is thus quite high. To obtain kinetic parameters for the monomer dissociation–reassociation, we have made use of slight charge differences between wild-type non-glycosylated SCF on the one hand, and other forms, i.e., N10D variant or wild-type glycosylated, on the other hand. Cation-exchange h.p.l.c. provides a rapid and convenient method for separating the differently charged forms from each other, such that rates of heterodimer formation (dimer hybridization) can be followed.

With sufficient incubation time after mixing, the presence of the 1:2:1 ratio of forms clearly shows that equilibrium in monomer dissociation–reassociation is reached. This is the case for both of the mixtures studied, indicating that there is no preference for self-association of any particular form (wild-type non-glycosylated, N10D variant or wild-type glycosylated). These data indicate that neither mutation to change charged state nor addition of bulky carbohydrate moieties on the amino acid side chains can have any significant effect on the SCF dissociation–association process. It is thus evident to predict that sequence regions near the N-terminus and the N- and O-linked glycosylation sites (i.e., Asn-65, Asn-109, Asn-120, Ser-142, Thr-143, and Thr-155) [23,24] are not at the SCF dimer contact area.

By following the rate of heterodimer formation, it is possible to arrive at a dissociation rate constant for dimerization using the equations derived in the Materials and methods section. This dissociation rate constant is large enough for substantial hetero-

dimer formation to occur within minutes after mixing. We cannot arrive at values for the association rate constant k_1 or for the equilibrium constant, $K_{eq.} (= k_1/k_{-1})$. However, under the present experimental conditions free monomer is not present at detectable levels, k_1 must be substantially greater than k_{-1} , such that dissociated monomers reassociate into dimers very rapidly. *In vitro*, SCF is active at the concentration in the ng/ml range [30] and soluble SCF concentration is found in the circulation at about 3 ng/ml [25]. These concentrations are substantially lower than those used in the studies described here. If $K_{eq.}$ is low enough, an appreciable amount of monomeric SCF may exist at physiological concentration. In this case, SCF monomer may exhibit biological activity and become physiologically important. Since we do not have a value for $K_{eq.}$, we cannot predict how much monomer might be present at the lower concentrations. This question together with the relevance of dimer dissociation-association for the biological significance of SCF will require further study.

In order that the determination of v_0 for heterodimer formation be accurate, the separation of forms during the cation-exchange h.p.l.c. must be rapid relative to v_0 . Indeed, the total running time of 15–30 min for the cation exchange is fairly long in comparison to the v_0 values determined. However, the fact that the amount of heterodimer detected in zero-time analyses is very small (Figure 2a) indicates that heterodimer formation during chromatography is minimal.

Typically, the monomeric components of oligomeric proteins are bound by van der Waal's contacts, hydrogen-bonding and electrostatic interactions [36,37]. A well-known example of subunit association-dissociation is that of lactate dehydrogenase, which can exist as heart (H) homotetramer, muscle (M) homotetramer, and as various hybrid (H-M) tetramers. In this case, the association constant is extremely high, with perturbing conditions, such as freezing-thawing being required to initiate hybridization of homotetramers [38,39]. In the case of SCF, freezing-thawing did not change the rate of dimer hybridization (results not shown).

Compared with the dissociation rate, k_{-1} , at pH 4.5, that at pH 3.2 was higher, while that at pH 8.2 was lower. Higher ionic strength resulted in a slightly increased k_{-1} . These effects of pH and ionic strength suggest that electrostatic interactions may be present at the monomer-monomer interface. For example, protonation of a carboxy group at pH 3.2 could decrease electrostatic interaction with a basic group and enhance the dissociation rate.

Such electrostatic interactions, along with van der Waal's contacts, hydrogen-bonding and hydrophobic interactions, contribute to the activation energy (E_a) of 85.6 kJ/mol (20.47 kcal/mol) determined for SCF monomer dissociation. This value is similar to the free energies of subunit association estimated for other oligomeric proteins [40].

SCF is analogous in many ways to two other growth factors, namely PDGF and, in particular, M-CSF. All three are dimeric [29,31–33] and their receptors (kit, PDGF receptor and *fms* respectively) are related members of the type III tyrosine kinase family [27,28]. The exon structures of the SCF and M-CSF genes are very similar, encoding extracellular, transmembrane and cytoplasmic domains [41]. Both proteins can be synthesized in forms that differ by the presence or absence, determined by alternative mRNA splicing, of amino acids encoded by the sixth exon; for both SCF and M-CSF, this region is on the extracellular side of the transmembrane domain and includes a proteolytic cleavage site such that the form containing the region tends to be processed, with release of soluble active factor corresponding to much of the extracellular domain [21,23,42]. The soluble SCF

and M-CSF are high in α -helix content [29,43], have homologous intrachain disulphide bonds, and it has been proposed that they have homologous four-helix bundle structures [41]. The molecular structure of the M-CSF dimer, determined by X-ray crystallography, has recently been reported [43]. The structure consists of the two monomers as four helix bundles laid end-to-end. Unlike the SCF dimer, the M-CSF dimer (and the PDGF dimer as well) is covalently held by an interchain disulphide bond (Cys³¹-Cys³¹) which resides in the large monomer-monomer contact region. The contact region also clearly has two reciprocal salt bridges involving Glu²⁸ and Arg⁶⁸ residues of the two monomers. It is likely that the structure within the SCF monomer-monomer contact region is similar to that for M-CSF, except for the disulphide bonding.

Like many receptors, including the PDGF and M-CSF receptors, kit undergoes dimerization on ligand binding [44,45]. For kit, this dimerization is probably mediated, at least to some extent [45], by the dimeric structure of SCF. An understanding of this dimeric structure, and of the dissociation-reassociation phenomena described here, will contribute to an understanding of the mechanisms of kit-receptor dimerization and signal transduction.

We are indebted to Ms. Joan Bennett for typing this manuscript, to Ms. Jette Wypych for providing glycosylated SCF, and to Dr. Tim Osslund in the Department of Molecular Structure, Amgen Inc., for his help in viewing the M-CSF monomer-monomer interface. W.-C. C. is Amgen's visiting scientist from the Institute of Biological Chemistry, Academia Sinica, and Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan.

REFERENCES

- Zsebo, K. M., Wypych, J., McNiece, I. K., Lu, H. S., Smith, K. A., Karkare, S. B., Sachdev, R. K., Yuschenkoff, V. N., Birkett, N. C., Williams, L. R. et al. (1990) *Cell* **63**, 195–201
- Martin, F. H., Suggs, S. V., Langley, K. E., Lu, H. S., Ting, J., Okino, K. H., Morris, C. F., McNiece, I. K., Jacobsen, F. W., Mendiaz, E. A. et al. (1990) *Cell* **63**, 203–211
- Zsebo, K. M., Williams, D. A., Geissler, E. N., Broudy, V. C., Martin, F. H., Atkins, H. L., Hsu, R.-Y., Birkett, N. C., Okino, K. H., Murdock, D. C. et al. (1990) *Cell* **63**, 213–224
- Williams, D. E., Eisenman, J., Baird, A., Rauch, C., Van Ness, K., March, C. J., Park, L. S., Martin, U., Mochizuki, D. Y., Boswell, H. S. et al. (1990) *Cell* **63**, 167–174
- Copeland, N. G., Gilbert, D. J., Cho, B. C., Donovan, P. J., Jenkins, N. A., Cosman, D., Anderson, D., Lyman, S. D. and Williams, D. E. (1990) *Cell* **63**, 175–183
- Huang, E., Nocka, K., Beier, D. R., Chu, Y.-Y., Buck, J., Lahm, H.-W., Wellner, D., Leder, P. and Besmer, P. (1990) *Cell* **63**, 225–233
- Russell, E. S. (1979) *Adv. Genet.* **20**, 357–459
- Silvers, W. K. (1979) *The Coat Colors of Mice: a Model for Mammalian Gene Action and Interaction*, Springer-Verlag, New York
- Chabot, B., Stephenson, D. A., Chapman, V. M., Besmer, P. and Bernstein, A. (1989) *Nature (London)* **335**, 88–89
- Geissler, E. N., Ryan, M. A. and Housman, D. E. (1988) *Cell* **55**, 185–192
- Anderson, D. M., Lyman, S. D., Baird, A., Wignall, J. M., Eisenman, J., Rauch, C., March, C. J., Boswell, H. S., Gimpel, S. D., Cosman, D. and Williams, D. E. (1990) *Cell* **63**, 235–243
- Nocka, K., Buck, J., Levi, E. and Besmer, P. (1990) *EMBO J.* **9**, 3287–3294
- McNiece, I. K., Langley, K. E. and Zsebo, K. M. (1991) *Exp. Hematol.* **19**, 226–231
- McNiece, I. K., Langley, K. E. and Zsebo, K. M. (1991) *J. Immunol.* **146**, 3785–3790
- Bernstein, I. D., Andrews, R. G. and Zsebo, K. M. (1991) *Blood* **77**, 2316–2321
- Broxmeyer, H. E., Hangoc, G., Cooper, S., Anderson, D., Cosman, D., Lyman, S. D. and Williams, D. E. (1991) *Exp. Hematol.* **19**, 143–146
- Andrews, R. G., Knitter, G. H., Bartelmez, S. H., Langley, K. E., Farrar, D., Hendren, R. W., Appelbaum, F. R., Bernstein, I. D. and Zsebo, K. M. (1991) *Blood* **78**, 1975–1980
- Andrews, R. G., Bartelmez, S. H., Knitter, G. H., Myerson, D., Bernstein, I. D., Appelbaum, F. R. and Zsebo, K. M. (1992) *Blood* **80**, 920–927
- Molineaux, G., Migdalska, A., Szmikowski, M., Zsebo, K. and Dexter, T. M. (1991) *Blood* **78**, 961–966
- Briddell, R. A., Hartley, C. A., Smith, K. A. and McNiece, I. K. (1993) *Blood* **83**, 1720–1723
- Flanagan, J. G., Chan, D. C. and Leder, P. (1991) *Cell* **64**, 1025–1035

- 22 Toksoz, D., Zsebo, K. M., Smith, K. A., Hu, S., Brankow, D., Suggs, S. V., Martin, F. H. and Williams, D. A. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7350–7354
- 23 Lu, H. S., Clogston, C. L., Parker, V. P., Wypych, J., Lee, T. D., Swiderek, K., Baltera, R. F., Jr., Patel, A. C., Brankow, D. W., Liu, X.-D., et al. (1992) *Arch. Biochem. Biophys.* **298**, 150–158
- 24 Lu, H. S., Clogston, C. L., Wypych, J., Fausset, P. R., Lauren, S., Mendiaz, E. A., Zsebo, K. M. and Langley, K. E. (1991) *J. Biol. Chem.* **266**, 8102–8107
- 25 Langley, K. E., Bennett, L. G., Wypych, J., Yancik, S. A., Liu, X.-D., Westcott, K. R., Chang, D. G., Smith, K. A. and Zsebo, K. M. (1993) *Blood* **81**, 656–660
- 26 Yarden, Y., Kuang, W.-J., Yang-Feng, T., Coussens, L., Munemitsu, S., Dull, T. J., Chen, E., Schlessinger, J., Francke, U. and Ullrich, A. (1987) *EMBO J.* **6**, 3341–3351
- 27 Ullrich, A. and Schlessinger, J. (1990) *Cell* **61**, 203–212
- 28 Miyajima, A., Kitamura, T., Harada, N., Yokota, T. and Arai, K.-I. (1992) *Annu. Rev. Immunol.* **10**, 295–331
- 29 Arakawa, T., Yphantis, D. A., Lary, J. W., Narhi, L. O., Lu, H. S., Prestrelski, S. J., Clogston, C. L., Zsebo, K. M., Mendiaz, E. A., Wypych, J. and Langley, K. E. (1991) *J. Biol. Chem.* **266**, 18942–18948
- 30 Langley, K. E., Wypych, J., Mendiaz, E. A., Clogston, C. L., Parker, V. P., Farrar, D. H., Brothers, M. O., Satyagal, V. N., Leslie, I., Birkett, N. C., Smith, K. A., Baltera, R. F., Jr., Lyons, D. E., Hogan, J. M., Crandall, C., Boone, T. C., Pope, J. A., Karkare, S. B., Zsebo, K. M., Sachdev, R. K. and Lu, H. S. (1992) *Arch. Biochem. Biophys.* **295**, 21–28
- 31 Johnsson, A., Heldin, C.-H., Westermark, B. and Wasteson, A. (1982) *Biochem. Biophys. Res. Commun.* **104**, 66–74
- 32 Das, S. K. and Stanley, E. R. (1982) *J. Biol. Chem.* **257**, 13679–13684
- 33 Glocker, M. O., Arbogast, B., Schreurs, J. and Deinzer, M. L. (1993) *Biochemistry* **32**, 482–488
- 34 Langley, K. E., Mendiaz, E. A., Liu, N., Narhi, L. O., Zeni, L., Parseghian, C. M., Clogston, C. L., Leslie, I., Pope, J. A., Lu, H. S., Zsebo, K. M. and Boone, T. C. (1994) *Arch. Biochem. Biophys.* **311**, 55–61
- 35 Clogston, C. L., Hsu, Y.-R., Boone, T. C. and Lu, H. S. (1992) *Anal. Biochem.* **202**, 375–383
- 36 Liljas, A. and Rossman, M. G. (1974) *Annu. Rev. Biochem.* **43**, 476–507
- 37 Jaenicke, R. (1982) *Biophys. Struct. Mech.* **8**, 231–256
- 38 Salter, S. N., Chilson, O. P. and Kaplan, N. O. (1965) *Nature (London)* **207**, 723–726
- 39 Chilson, O. P., Costello, L. A. and Kaplan, N. O. (1965) *Biochemistry* **4**, 271–281
- 40 Chothia, C. and Janin, J. (1975) *Nature (London)* **256**, 705–708
- 41 Bazan, J. F. (1991) *Cell* **65**, 9–10
- 42 Rettenmier, C. W. (1989) *Curr. Top. Microbiol. Immunol.* **149**, 129–141
- 43 Pandit, J., Bothm, A., Jancarik, J., Halenbeck, R., Kothe, K. and Kim, S.-H. (1993) *Science* **258**, 1358–1362
- 44 Blume-Jensen, P., Claesson-Welsh, L., Siegbahn, A., Zsebo, K. M., Westermark, B. and Heldin, C.-H. (1991) *EMBO J.* **10**, 4121–4128
- 45 Lev, S., Yarden, Y. and Givol, D. (1992) *J. Biol. Chem.* **267**, 15970–15977