Calcium-dependent Complex Assembly of the Myeloic Differentiation Proteins MRP-8 and MRP-14*

(Received for publication, February 21, 1991)

Stefan Teigelkamp, Ranjit S. Bhardwaj, Johannes Roth, Georg Meinardus-Hager, Michael Karas‡, and Clemens Sorg§

From the Institute of Experimental Dermatology and the Institute of Medical Physics, University of Münster, D-4400 Münster, Germany

MRP-8 and MRP-14 are calcium-binding proteins belonging to the S-100 protein family which have been shown to be associated with specific stages of myeloic/monocytic cell differentiation. Members of this protein family are shown to form homo- and heterodimers. Complex formation has also been observed in preliminary experiments for MRP-8 and MRP-14. To evaluate the in vivo relevance of the MRP complex formation and the stoichiometric ratio of individual components complexes were isolated from granulocytes and monocytes by immunofluorescence chromatography using monospecific antibodies. The purified fraction of the MRPs was found to contain monomers and dimers as shown on sodium dodecyl sulfate-polyacrylamide gel electrophoresis by silver staining and immunoblotting. Similar results were obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting of crude cell extracts. The existence of the MRP complexes in vivo was demonstrated by chemical cross-linking and subsequent isolation of complexes by immunofluorescence chromatography. Two new, highly abundant complexes were found in addition to the heterodimer, but neither monomers nor homodimers were detected. The two larger protein complexes (35.0 and 48.5 kDa) were identified as ((MRP-8)_2*(MRP-14)) trimer and ((MRP-8)_2*(MRP-14)_2) tetramer, respectively. All complexes could be shown to be noncovalently associated in vivo. Furthermore, the association of MRPs was shown to be Ca** dependent.

The two proteins MRP-8 and MRP-14 have recently been isolated and molecularly cloned (1, 2). It was shown that the MRP-14 was largely identical with the so-called cystic fibrosis antigen (3, 4), and both proteins were identical with the so-called light and heavy chain of the L1 antigen which has been found in granulocytes (5). The expression of the proteins is not confined to granulocytes. They are also expressed in monocytes but disappear in mature forms of macrophages (6).

Although the proteins are generally expressed by infiltrating monocytes but disappear in mature forms of macrophages (6). Although no definite function could be assigned for this protein family there is evidence that they are involved in cell cycle progression, cell differentiation, cytoskeletal membrane interaction, and phosphorylation events (9, 10). Recent studies have shown an inhibitory activity of a complexed form of MRP-8 and MRP-14 for casein kinases I and II (11).

From the cited work and our own data it seems obvious that the two molecules are coexpressed by the same cell, and a great part of the functions may be exerted by complexes of the two molecules. However, the composition of the complexes including participation of other molecules in complex formation has not been studied thoroughly. In addition it is unclear whether different MRP-8-MRP-14 complexes exist in vivo and what function they exert with respect to different phenotypic functions of e.g. the respective macromolecules.

In the present paper we demonstrate three distinct complexes and their stoichiometry as the predominant form of intracellular MRP-8 and MRP-14. No third component was detected. The complexes were noncovalently associated in dependence of the calcium concentration.

EXPERIMENTAL PROCEDURES

Preparation of Monocyte and Granulocyte Extracts—Monocytes and granulocytes were isolated from human buffy coat by ficoll-paque (Pharmacia, Uppsala) density gradient centrifugation (12, 13). Purity of monocytes and granulocytes was >90% in all experiments as obtained by immunocytological techniques using cell-specific monoclonal antibodies directed against CD14 (Immunotech, via Dianova and Dako, Hamburg, Germany), CD15, and CD16 (Immunotech). The purified monocytes as well as granulocytes were resuspended in phosphate-buffered saline (PBS), pH 7.6, for cross-linking experiments or Tris-buffered saline (TBS). pH 7.6, for purification of MRPs. The cells were disrupted by repeated freezing-thawing (three times) and subsequent nitrogen cavitation (20 min at 0°C and 30 bar). The suspensions were centrifuged at 17,000 × g to remove cell debris. The supernatant was filtered through a 0.22-μm membrane and stored at

* This work was supported by grants from the Bundesministerium fur Forschung und Technologie. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

† To whom correspondence should be sent: Institute of Experimental Dermatology, University of Münster, von-Esmarch-Strasse 56, D-4400 Münster, Germany; Tel.: 0251-83657; Fax: 0251-89536.

‡ The abbreviations used are: PBS, phosphate-buffered saline; TBS, Tris-buffered saline; BS**; bis-(sulfosuccinimidyl)propionate; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; LDI, laser desorption/ionization; rMRP, recombinant MRP.
from Monocytes
the reactions were quenched by addition of 0.166 volume of 50 mM
by matrix-assisted laser desorption/ionization (LDI) (18, 19). Sam-
were prepared in the same concentrations as described above. A 20
prepared as described above in PBS containing either 10 or 40 mM
eluted with 0.1 M glycine HCl, pH 2.5. Fractions containing protein
were loaded onto the immunoaffinity column, washed with TBS, and
stored at -80 °C.
Cross-linking of MRPs—The cross-linking of MRP complexes was
 carried out by using BS' (bis-(sulfosuccinimidyl)dianilino) or DTSSP (3,3'-dithiobis(sulfosuccinimidylpropionate)) (Pierce Chem-
ical Co.), according to Stros (14). Briefly, affinity-purified MRPs
(see above) were diluted in PBS, pH 7.4, to a concentration of 0.5,
0.25, and 0.125 mg/ml. Crude extracts of granulocytes and monocytes
were prepared in the same concentrations as described above. A 20
mM stock solution of cross-linker was prepared shortly before use.
Protein samples were treated with various concentrations of cross-
linkers (0.05-4.5 mM) for 30 min at room temperature. Subsequently,
the reactions were quenched by addition of 0.166 volume of 50 mM
ethanamine, 20 mM N-ethylmaleimide, and 50 mM sodium phos-
phate, pH 7.4, and then stored at -80 °C. Samples cross-linked with
BS' were incubated with 1% β-mercaptoethanol before separation on
SDS-gel electrophoresis.
Immunostaining of MRPs on Western Blots—Supernatants of cell
lysates, cross-linked with BS' or DTSSP, were separated on SDS-
PAGE (15) and semidry blotted (16) onto polyvinylidene difluoride
membrane (Immobilon P, Millipore, Eschborn, Germany).
Non-specific binding of antibodies was blocked by immersion of
the membranes in 1% milk powder in PBS at 4 °C overnight. Subse-
quent, a 1-h incubation with anti-rMRP-8 and anti-rMRP-14 antibo-
dy was carried out (0.5 µg/ml anti-rMRPs in PBS containing 1%
bovine serum albumin). After several washes in PBS, 0.05% Tween
20, the bound antibodies were detected by affinity-purified goat anti
rabbit alkaline phosphatase-conjugated antibodies (Dianova). Again,
the membranes were washed extensively in TBS, 0.05% Tween, and
then Tris-HCl, pH 9.5, 0.1 M NaCl, 50 mM MgCl2, 0.05% Tween.
Alkaline phosphatase activity was revealed by developing the mem-
branes in a solution containing 0.15 mg/ml 5-bromo-4-chloro-3-
indoylphosphate (Sigma) and 0.5 mg/ml nitroblue tetrazolium chlo-
ride (Sigma) in 0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl, 50 mM MgCl2,
0.05% Tween 20.
Silver Staining of SDS-PAGE—Affinity-purified MRP complexes,
separated on SDS-PAGE, were silver stained by the method of
Ansgore (method A) (17).
A modified silver staining (method B) for subsequent densitometric
quantitation of the protein bands was carried out as follows. After
fixation and washing with H2O gels were rinsed in a 0.15% solution
of AgNO3 for 30 min, washed in H2O for 25 s, and developed in 2.5%
Na2CO3, 0.01% formaldehyde. The reaction was stopped by incuba-
tion in 50 mM EDTA. The gels were slightly destained with 0.2%
Farmer's reagent and then washed extensively with H2O. Quantifi-
cation of protein bands was carried out on a laser densitometer (Ultra
Scan XL, Pharmacia).
Additional Methods—Mass spectroscopy of MRPs was carried out
by matrix-assisted laser desorption/ionization (LDI) (18, 19). Sample
supernatants were diluted to a concentration of 0.1 mg/ml in H2O and then
mixed with a solution of 50 mM nicotinic acid in a 1:1 ratio, dried onto
a metallic substrate, and transferred to the vacuum chamber of
a time-of-flight mass spectrometer.
Enzyme-linked immunosorbent assay was performed according to
Bruggen and Sorg (20).
For alkylation of free thiol groups supernatants of cell lysates were
prepared as described above in PBS containing either 10 or 40 mM
iodoacetamide (21). After a 45-min incubation at room temperature,
unbound iodoacetamide was removed by Sephadex G-25 gel filtration
(PD-10, Pharmacia) and prepared for SDS-PAGE as usual.
RESULTS
Purification and Characterization of MRP-8 and MRP-14 from Monocytes and Granulocytes—As a source for MRP-8
and MRP-14 purification, monocytes and granulocytes were
isolated from human buffy coats. Cells were disrupted by
nitrogen cavitation and centrifuged. The supernatants thus
obtained from granulocytes and monocytes contained 4–6 mg/
ml total protein (Bradford method). The content of MRPs
differed as determined by enzyme-linked immunosorbent assay
and was 5–10% of the total protein content of granulocyte
lysate supernatants; levels of MRPs in monocyte lysate super-
natants were found to be 20–50 times lower. This super-
natant was loaded on an affinity column with monospecific
anti-rMRP-14 antibodies. The eluate was analyzed for the presence
of MRPs by mass spectroscopy and silver staining after
SDS-PAGE was carried out under reducing and nonre-
ducing conditions (Fig. 1, A and B). Under reducing condi-
tions only two bands were found representing MRP-8 and
MRP-14 monomers. Nonreducing conditions revealed dimeric
forms, indicating that cysteine linkage is responsible for their
existence (Fig. 1B). Western blot analysis with monospecific
antisera showed that the band at 22.0 kDa is an MRP-8
homodimer; at 24.5 kDa, an MRP-8-MRP-14 heterodimer;
and at 26.5 kDa, an MRP-14 homodimer (Fig. 1C).
To determine the molecular weight of MRP-8 and MRP-
14 more precisely, anti-rMRP-14 antibody affinity-purified
MRPs were measured by matrix-assisted LDI mass spectro-
copy. It could be shown that MRP-8 has a molecular mass
of 11,007 ± 33 Da (n = 3). For MRP-14 two molecular masses
were distinguished, namely 12,878 ± 61 Da (n = 3) and 13,288

FIG. 1. Analysis of affinity-purified MRP-8 and MRP-14.
A, typical LDI mass spectrum of affinity-purified MRPs. No peaks
corresponding to molecular masses > 25,000 Da were found. B,
analysis of affinity-purified MRPs on SDS-PAGE (15%) under lane
a, restring (1% β-mercaptoethanol) and lane b, nonreducing condi-
tions (silver stain, method A). C, Western blotting of affinity-purified
MRPs and subsequent immunostaining with lane c, anti-rMRP-8 and
lane d, anti-rMRP-14 monospecific antisera.
purified by an anti-rMRP-14 affinity column were used. Therefore, for further investigation, as a source for MRP-8 and MRP-14 molecules lysates from human granulocytes, expect to obtain MRP-8 monomers and homodimers from an anti-rMRP-14 affinity column, and vice versa. This, however, is different from our results obtained in immunoblot experiments (Fig. 1C). A likely explanation could be that both proteins form noncovalently linked complexes that disintegrate during SDS-PAGE. Therefore, cross-linking experiments employing Bis(sulfosuccinimidyl) suberate (BS") were carried out. Upon cross-linking of affinity purified MRPs and subsequent separation on SDS-PAGE under reducing conditions, two new protein bands of 35.0 and 48.5 kDa (Fig. 2A) in addition to the MRP-8-MRP-14 heterodimer (24.5 kDa) were detected by silver staining. To evaluate the specificity of the cross-linking procedure, experiments were performed with different MRP concentrations. In parallel, cross-linking was carried out in supernatants prepared from monocyte and granulocyte lysates, separated by SDS-PAGE, and electroblotted. Blots were stained with anti-rMRP-14 or anti-rMRP-14 antibodies. Each of these experiments exhibited the identical pattern of MRP complex formation (Fig. 2B) as was apparent in the affinity-purified fractions. This confirms the complex formation in vivo. Further, to show the specificity of the cross-linking procedure, the cross-linking reagent was employed at a concentration even 5-10 times higher than recommended by Staros (14). Again, previous results were reproduced, and no larger complexes were seen (Fig. 2B). Interestingly, monomers and homodimers were either absent or only very weakly stained (Fig. 2).

Composition of the MRP-8-MRP-14 Complexes—The further attempt was to evaluate the composition of the complexes described above. To evaluate the possibility of the existence of a third component in the large complexes, an affinity-purified MRP fraction was split into two aliquots. One of the aliquots was cross-linked. Both were separated under reducing conditions on SDS-PAGE and visualized by silver staining (method A). In the cross-linked aliquot the very prominent 35.0- and 48.5-kDa band and a weak 24.5-kDa band were detected whereas in the other aliquot only MRP-8 and MRP-14 monomers could be seen. These results indicate that MRP-8 and MRP-14 are the only molecules involved in complex formation. Because of the apparent molecular weight of the two new protein bands, the 35.0- and 48.5-kDa complex represent a trimer and a tetramer, respectively, whereas the 24.5-kDa complex is a heterodimer, as described above. To determine the stoichiometric ratio of the single components in the trimer and the tetramer, the following experiment was performed. The affinity-purified MRP complexes were cross-linked with DTSSP, a cross-linker containing a \( \beta \)-mercaptoethanol-cleavable disulfide bond. The complexes were separated on SDS-PAGE under nonreducing conditions. Each single protein band, corresponding to its molecular weight, was excised from the gel and boiled for 10 min in SDS-PAGE sample buffer containing 5% \( \beta \)-mercaptoethanol for cleavage of cross-linkers. Subsequently, each gel patch was placed onto a second SDS gel to separate the now existing monomers. Protein bands were detected by silver staining (method B), and the intensity of the stained bands was determined densitometrically with a laser scan densitometer. Integration of peak areas indicated that the 35.0-kDa protein is an ([MRP-8]_12-MRP-14) trimer and the 48.5-kDa protein is an ([MRP-8]_1-MRP-14)_2 tetramer (Fig. 3). However, the possibility that both MRP-14 isomers mentioned above are involved in the complex formation cannot be ruled out (see "Discussion").

Noncovalent Association of MRP Complexes—The results shown above indicate the presence of a disulfide bridge in the protein complexes. To determine whether the disulfide bond found in the heterodimer is of in vivo relevance, a granulocyte lysate was prepared in PBS containing different amounts of the free thiol group blocker iodoacetamide. Samples were

---

**Fig. 2. Cross-linking of MRP-8-MRP-14 complexes.** A, 0.5 mg/ml affinity-purified MRPs from granulocyte lysate were cross-linked with the aid of the cross-linker BS": lane a, none; lane b, 0.05 mM; lane c, 0.10 mM; lane d, 0.25 mM; and lane e, 0.50 mM BS". Samples were subjected to SDS-PAGE (15%) under reducing conditions (1% \( \beta \)-mercaptoethanol). Subsequently, protein bands were visualized by silver staining (method A). B, staining pattern of protein bands obtained after cross-linking in granulocyte lysate, separation on SDS-PAGE, and subsequent immunostaining of the Western blots with anti-rMRP-8 (left lanes a-g) and anti-rMRP-14 (right lanes a-g) antisera. Concentrations of lane a, none; lane e, 0.5 mM; lane f, 1.5 mM; and lane g, 4.5 mM BS" were used in this experiment.
MRP-8 and MRP-14 Complex Formation

FIG. 3. Densitometric evaluation of the stoichiometric composition of native MRP complexes. The relative amounts of the MRP-8 (1) and MRP-14 (2) component in the dimer (24.5 kDa), trimer (35.0 kDa), and tetramer (48.5 kDa) were determined by densitometric scanning of silver-stained polyacrylamide gels (method B). A indicates the percentage of the appropriate peak areas (peak 1 + peak 2 = 100%) used for the evaluation of the stoichiometric composition shown in B.

Fig. 4. Alkylation of free thiol groups by iodoacetamide treatment. Granulocyte lysates were incubated with lanes a and d, none; lanes b and e, 10 mM; and lanes c and f, 40 mM iodoacetamide (45 min at room temperature). Samples were separated on SDS-PAGE (15%) under nonreducing conditions without cross-linking (lanes a–c) and after cross-linking with 1.5 mM BS² (lanes d–f) and subsequently electroblotted. Western blots were stained with A, anti-rMRP-8 and B, anti-rMRP-14 antisera.
MRP-8 and MRP-14 Complex Formation

**Fig. 5.** Ca\(^{2+}\)-dependent complex formation of MRP-8 and MRP-14. Granulocyte lysate (0.25 mg/ml) in PBS containing lane a, 1.0 mM; lane b, 0.1 mM; lane c, 0.01 mM; and lane d, 0.001 mM EDTA; lane e, none (EDTA/Ca\(^{2+}\)); lane f, 0.001 mM; lane g, 0.01 mM; lane h, 0.1 mM; and lane i, 1.0 mM Ca\(^{2+}\) was cross-linked with BS\(^{3}\). Samples were separated on SDS-PAGE (15%) and subsequently electroblotted. Western blots were stained with A, anti-rMRP-8 and B, anti-rMRP-14 antisera.

RESULTS

Previously we have described two Ca\(^{2+}\)-binding proteins associated with defined stages of myeloid cell differentiation. The amino acid sequence of MRP-8 and MRP-14 (1, 6), derived from cDNA studies, revealed that MRP-8 contains 93 amino acids and MRP-14 114 amino acids. Molecular weights of MRPs predicted from amino acid composition derived from cDNA studies ignores post-translational modifications. Applying SDS-PAGE, different investigators have assigned different molecular weights to the native molecules (22–24). Therefore we used matrix-assisted LDI mass spectroscopy, which has been shown to provide a far more exact molecular weight than the methods mentioned above (18, 19). Here we found molecular masses of 11,007 ± 33 Da for MRP-8 and a doublet of clearly distinguishable molecular masses of 12,878 ± 61 Da and 13,282 ± 55 Da corresponding to MRP-14 (calculated molecular mass for MRP-8 was 10,835 Da and for MRP-14, 13,242 Da). The possibility that one of these doublet peaks arose from a contaminating protein could be excluded by two-dimensional gel electrophoresis and subsequent immunoblotting with anti-rMRP-14 antibodies. These results indicate the existence of two forms of MRP-14. Similar results have also been reported by Berntzen and Fagerhol (23) and Edgeworth et al. (25). Furthermore, the latter group reported a post-translational phosphorylation of both of these MRP-14 proteins (25). No other post-translational modification is known except an N-terminal acylation of MRP-14 (26). These known post-translational modifications could not primarily be responsible for creating the 410 ± 46 Da difference in molecular mass of MRP-14, as shown by LDI measurements. This led us to the assumption that the molecule of lower mass is a truncated form of MRP-14 (named here MRP-14'). The obvious difference in molecular mass, as is evident from LDI measurements, indicates the absence of approximately four amino acids in MRP-14' compared with MRP-14. Presumably, MRP-14' is a product of post-translational proteolytic cleavage of MRP-14. However, the amino acid sequence revealed by cDNA analysis shows a second methionine in position 5 of MRP-14: MTCKMQL (single-letter amino acid code) (1, 2). Consequently, the ATG coding for the methionine at position 5 may also function as a start codon, as it is shown for some other proteins (27–29). Alternative splicing as a cause for the existence of the two isoforms may not be likely because only full-length cDNA clones have been found (1), which could merely be derived from one mRNA species.

In addition to the distinction of MRP-14 and MRP-14', we could also detect a peak at 22.4 kDa representing a heterodimer of MRP-8 and MRP-14. The heterodimer could also be visualized by immunoblotting with anti-rMRP-8 and anti-rMRP-14 antibodies. This shows that both molecules are able to form complexes. The question of whether larger complexes of these proteins exist arose from affinity purification of the MRPs, as we resolved the identical elution pattern with both of the applied nonspecific antibodies. The methods employed in this study do not preserve complexes stabilized only by noncovalent interactions. Therefore chemical cross-linking was applied immediately after cell disruption to exclude decomposition of complexes. The results derived from these experiments revealed a 48.5-, a 35.0-, and a 24.5-kDa band, as detected by immunoblotting. These protein complexes are demonstrated to be the predominant intracellular forms of the MRPs. Further, the densitometric analysis illustrated the 35.0-kDa band as a trimeric form, composed of ((MRP-8)?-(MRP-14)) and the 48.5-kDa band as a tetrameric form of ((MRP-8)?-(MRP-14)). These experiments did not indicate whether MRP-14' participates in trimer and tetramer formation. However, the results of Berntzen and Fagerhol (23), which show that MRP-14' (L-1 heavy chain I) is one of the three subunits of the L-1 complex (23), obviously corresponding to our trimer, indicate involvement of MRP-14 and MRP-14' in trimer formation ((MRP-8)?-(MRP-14)) and ((MRP-8)?-(MRP-14')).

Further investigation was undertaken to provide an explanation for how the complexes are assembled. The detection of purified MRPs as dimers as well as monomers on SDS-PAGE under nonreducing conditions indicated that cysteine bridges are involved in complex formation. However, blocking of free thiol groups with iodoacetamide revealed only monomeric forms, demonstrating no disulfide bonds among these proteins. Cross-linking the iodoacetamide-treated samples

---

2 S. Teigelkamp, R. S. Bhardwaj, J. Roth, G. Meinardus-Hager, M. Karas, and C. Sorg, unpublished data.
showed the existence of the heterodimer (24.5 kDa), trimer (35.0 kDa), and tetramer (48.5 kDa). Hence, we conclude that \textit{in vivo} the MRPs form only noncovalently linked complexes. Oppositely, the related proteins S-100-\(\alpha\) (cysteine 85), S-100-\(\beta\) (cysteine 68 and 84), and p11 (cysteine 61 and 82) are shown to be disulfide bound (30–32). This difference, however, can easily be explained by the fact that MRP-8 and MRP-14 lack a cysteine residue in that part of the C-terminal half which has been proposed to participate in intermolecular connections (32). Evidently, the N-terminal cysteine residues in MRP-8 (cysteine 42) and MRP-14 (cysteine 3) do not take part in intermolecular disulfide bonds in the assembly of MRP complexes.

In further studies the influence of Ca\(^{2+}\) on complex formation was investigated. A decomposition of the MRP complexes in granulocyte/monocyte lysates as well as in affinity-purified eluate upon trapping Ca\(^{2+}\) by EDTA was shown. Evidently, the MRP complex assembly is a Ca\(^{2+}\)-regulated process. This observation allows us to speculate on a possible function of the MRPs as a modulator/regulator in the Ca\(^{2+}\) signal pathway, which could be executed by their complex formation/degradation. One of the potential candidates influenced by this proposed mechanism might be casein kinase I and II, which is reported to be inhibited by an MRP-8-MRP-14-containing complex (11).

This study demonstrates the existence of three different MRP-8 and MRP-14-containing complexes, the formation of which is Ca\(^{2+}\) dependent. Several studies of our group demonstrated up-regulation of MRP-8 and MRP-14 in myeloid cells in the course of inflammatory events (1, 6, 33). Preliminary evidence suggests that the expression of MRP-8 and MRP-14, which are both coded for on chromosome 13 (4), are under separate control. Although both proteins are expressed by monocytes/macrophages in chronic inflammatory processes such as rheumatoid arthritis or sarcoidosis, a dissociation is observed in acute inflammation (1, 6, 7). Furthermore the assembly of the complexes seems to represent distinct steps in the maturation of monocytes in chronic inflammation although it is not seen in acute inflammation (6). Future studies therefore will be directed at the functional characterization of MRP-8- and MRP-14-expressing monocytes/macrophages. The clearcut differences to the differentiation patterns in chronic inflammation might provide clues as to the pathomechanisms of a variety of chronic inflammatory diseases.

Acknowledgments—We thank Dieter Wiesmann for excellent technical assistance and Brunhilde Scheibel for typing the manuscript.

L. Grün, unpublished data.

REFERENCES

1. Odink, K., Cerletti, N., Brüggen, J., Clerc, R. G., Tarsays, L., Zwaldlo, G., Gerhardas, G., Schlegel, R., and Sorg, C. (1987) Nature 330, 80–82
2. Lagasse, E., and Clerc, R. G. (1988) Mol. Cell. Biol. 8, 2402–2410
3. Brüggen, J., Tarsays, L., Cerletti, N., Odink, K., Rutishauer, M., Höländer, G., and Sorg, C. (1988) Nature 331, 570
4. Dorin, J. R., Novak, M., Hill, R. E., Brock, D. J. H., Secher, D. S., and van Heyningen, V. (1987) Nature 326, 614–617
5. Andersson, K. B., Sletten, K., Berner Bernsten, H., Fagerhol, M. K., Daim, I., Brandtzaeg, P., and Jellum, E. (1988) Nature 332, 13467
6. Zwaldlo, G., Brüggen, J., Gerhardas, G., Schlegel, R., and Sorg, C. (1988) Clin. Exp. Immuno. 72, 510–515
7. Delabie, J., De Wolf-Peeters, C., van den Oord, J., and Desmet, V. J. (1990) Clin. Exp. Immuno. 81, 125–126
8. Gabrielsen, T. O., Dale, J., Brandtzaeg, P., Hoei, P. S., Fagerhol, M. K., Larsen, T. E., and Thune, P. O. (1986) J. Am. Acad. Dermatol. 15, 173–179
9. Kligman, D., and Hilt, D. C. (1988) Trends Biochem. Sci. 13, 437–443
10. Persechini, A., Moncrief, N. D., and Kreitsinger, R. H. (1989) Trends Neurosci. 12, 462–467
11. Munro, S., Collart, F. R., and Huberman, E. (1989) J. Biol. Chem. 264, 8566–8560
12. Boyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, 77–89
13. Feige, U., Overwien, B., and Sorg, C. (1982) J. Immunol. Methods 54, 309–315
14. Staros, J. V. (1982) Biochemistry 21, 3950–3955
15. Laemmli, U. K. (1970) Nature 227, 680–685
16. Towbin, H., Staehlin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4550–4554
17. Ansorge, W. (1985) J. Biochem. Biophys. Methods 11, 13–20
18. Karas, M., and Bahr, U. (1990) Trends Anal. Chem. 9, 321–325
19. Hillenkamp, F., Karas, M., Ingendoh, A., and Stahl, B. (1990) in Biological Mass Spectrometry (Burlingame, A., and McCloskey, J. A., eds) pp. 49–90, Elsevier, Amsterdam
20. Brüggen, J., and Sorg, C. (1983) Cancer Immuno. Immuno. 15, 200–205
21. Creighton, T. E. (1988) in Protein Structure: A Practical Approach (Creighton, T. E., ed) pp. 155–166, IRL Press, Oxford
22. Dale, I., Fagerhol, M. K., and Naesgaard, I. (1988) Eur. J. Biochem. 134, 1–6
23. Bertzten, H. B., and Fagerhol, M. K. (1990) Scand. J. Clin. Lab. Invest. 50, 769–774
24. van Heyningen, V., and Dorin, J. (1990) Adv. Exp. Med. Biol. 269, 139–143
25. Edgeworth, J., Freemont, P., and Hogg, N. (1989) Nature 342, 189–192
26. Tohe, T., Murakami, K., Tomita, M., and Nozawa, R. (1989) Chem. Pharm. Bull. (Tokyo) 37, 1576–1580
27. Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148
28. Strubin, M., Long, E. O., and Mach, B. (1986) Cell 47, 619–625
29. Peterson, C. A., and Piatigorsky, J. (1986) Gene (Amst.) 49, 139–147
30. Baudier, J., and Cole, R. D. (1988) J. Biol. Chem. 263, 5876–5883
31. Baudier, J., and Cole, R. D. (1988) Biochemistry 27, 2728–2736
32. Johnson, N., and Weber, K. (1990) J. Biol. Chem. 265, 14464–14468
33. Roth, J., Sunderköttter, C., Teigelkamp, S., and Sorg, C. (1990) Immunobiology 181, 199
34. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254