Decay-accelerating Factor (DAF), Complement Receptor 1 (CR1), and Factor H Dissociate the Complement AP C3 Convertase (C3bBb) via Sites on the Type A Domain of Bb*

The AP C3 convertase, C3bBb(Mg\(^{2+}\)), is subject to irreversible dissociation (decay acceleration) by three proteins: DAF, CR1, and factor H. We have begun to map the factor B (fB) sites critical to these interactions. We generated a panel of fB mutations, focusing on the type A domain because it carries divalent cation and C3b-binding elements. C3bBb complexes were assembled with the mutants and subjected to decay acceleration. Two critical fB sites were identified with a structural model. 1) Several mutations centered at adjacent alpha helices 4 and 5 (Gln-335, Tyr-338, Ser-339, Asp-382) caused substantial resistance to DAF and CR1-mediated decay acceleration but not factor H. 2) Several mutations centered at the α helix and adjoining loops (especially D254G) caused resistance to decay acceleration mediated by all three regulators and also increased C3b-binding affinity and C3bBb stability. In the simplest interpretation of these results, DAF and CR1 directly interact with C3bBb at α 4/5; factor H likely interacts at some other location, possibly on the C3b subunit. Mutations at the C3bBb interface interfere with the normal dissociation of C3b from Bb, whether it is spontaneous or promoted by DAF, CR1, or factor H.

Complement activation can be initiated by three different pathways: the classical pathway (CP), the alternative pathway (AP), or the lectin pathway (1). Each initiation pathway functions in common to form C3 convertases, active serine proteases that amplify complement activation by cleaving the serum protein C3 into two fragments. One C3 fragment, C3a, is a anaphylactic agent, while the other fragment, C3b, binds covalently to activating targets, marking foreign substances for an anaphylactic agent, while the other fragment, C3b, binds covalently to activating targets, marking foreign substances for dissipation at the C3bBb interface interfere with the normal decay acceleration of these complexes by DAF, CR1, and factor H.

Activation of human C3 is regulated by a family of related proteins termed the regulators of complement activation (RCA) (5–7). The RCA proteins control complement activation by virtue of two different activities. 1) RCA proteins can promote the irreversible dissociation of complement convertases. 2) RCA proteins can serve as cofactors in the factor I-mediated cleavage of C3b and C4b. The AP C3 convertase, C3bBb(Mg\(^{2+}\)), is subject to decay acceleration by three RCA proteins: DAF, factor H, and CR1. These three regulators, like all other RCA proteins, are composed of arrays of tandem globular domains termed CCPs (complement control protein repeats) or SCR (short consensus repeats). Only a portion of each protein is necessary and sufficient for decay acceleration of C3bBb(Mg\(^{2+}\)): CCPs 2–4 of DAF (8), CCPs 1–3 of CR1 (9), and CCPs 1–4 of factor H (10). While the mechanism of decay acceleration is largely unknown, previous studies have indicated that DAF may interact with the Bb subunit during this process (11). In addition, it has been postulated that a positively charged surface area in DAF CCP 2 and CCP 3 is the primary C3 convertase recognition area (12).

Here, we sought to elucidate the mechanism of AP C3 convertase decay acceleration by mapping sites on the Bb subunit critical to decay acceleration. We previously developed a simple ELISA method for the investigation of decay acceleration of the AP C3 convertase (13). In this report we describe a panel of Bb mutations, their assembly into mutant C3bBb complexes, and the decay acceleration of these complexes by DAF, CR1, and factor H. Interpretation of the results with a structural model of the factor B type A domain implicates two Bb sites critical to DAF and CR1 mediated decay acceleration, one of which is not critical to factor H-mediated decay acceleration.

EXPERIMENTAL PROCEDURES

Production and Assessment of Mutant Factor B Proteins—The transient expression of mutant and wild type factor B proteins was conducted with a human factor B cDNA in pSG5 vector using human 293T kidney cells in serum-free medium (14). Mutations were introduced into the factor B clone using the QuikChange site-directed mutagenesis method (Stratagene).

Each mutant was assayed for its capacity to participate in C3bBb assembly by three assays. 1) A fluid phase assay was used to examine the C3b- and factor D-dependent cleavage of factor B (15). 2) An ELISA-
yielded consistent results. The percent change in average OD at 10 min, though analyses performed with the 20-min and 30-min time point comparison between the mutant and wild type factor B proteins, well. Analysis of the 10-min time point allowed the most sensitive bound C3bBb(Ni2

Procedures

ng/ml) in the presence of factor D (25 ng/ml), 2 mM NiCl2, 25 mM NaCl, and phosphate buffer supplemented with 4% BSA and 0.1% Tween 20, (Fig. 1). In each experiment C3bBb(Ni2

InsightII software platform, MSI.

Decay Acceleration Sites of the AP C3 Convertase

The data shown are the results of 29 different experiments with an average of three mutants per experiment (Fig. 1). In each experiment C3bBb(Ni2

Proteins—Complement proteins factor D, C3b, and factor H were obtained from Advanced Research Technologies, La Jolla, CA. DAF consisted of a soluble derivative containing the 4 CCP active region with a single amino acid substitution (N81Q) that removed the N-linked oligosaccharide situated between CCP 1 and 2 (17, 18). CR1-A is a soluble CR1 derivative, consisting of CCP 1–7 as previously described (19).

Protein Modeling—The factor B type A domain model was constructed with the spatial coordinates of the iC3b receptor (CR3) type A domain (20), PDB entry 1IDO using the alignment provided by Hopefsed et al. (21), the automated program Modeler (22), and the InsightII software platform, MSI.

Fig. 1. Decay Acceleration Assay (see “Experimental Procedures”).

Fig. 2. Model of the factor B type A domain. A, C3b-binding peptides are shaded. Residues that coordinate the divalent cation (Asp-251 (D251), Ser-253 (S253), Ser-255 (S255), Thr-328 (T328), Asp-364 (D364)) are marked by spheres. B, amino acid positions mutated in the study are marked by spheres.

RESULTS

Construction of a Panel of Factor B Type A Mutants—The Bb fragment consists essentially of two protein domains: a von Willibrand factor type A domain and a serine protease domain (23). From structural studies of type A domains of other proteins, the factor B type A domain is expected to be a globular structure composed of several parallel β sheets surrounded by seven a helices (20, 24). The three-dimensional coordinates of the type A domain of factor B have not been reported although spatial models can be produced based on the three-dimensional coordinates of other type A domains (21, 25). Thus, a spatial model was generated using the three-dimensional coordinates of the type A domain of CR3 that had been derived by x-ray crystallography. The CR3 domain was chosen because it binds the C3b derivative iC3b in the presence of Mg2

We constructed a panel of factor B type A mutants. Most mutations consisted of alanine substitutions for one or two charged or polar amino acids that are predicted to be on the protein’s exterior. According to our model, the mutations were distributed over much of the type A domain surface (Fig. 2B).
Among the recombinants produced were 31 mutant proteins that showed function in a factor D-dependent, divalent cation-dependent, C3b-binding assay and could be used to detect decay acceleration. Together, the mutations encompassed changes at 29 amino acid positions, including those of the two N-linked glycans.

Effects of Factor B Mutations on Decay Acceleration—Each factor B mutation was examined for its effects on the decay acceleration activity of DAF; mutant and wild type C3bBb(Ni2⁺) complexes were generated in C3b-coated microtiter wells. The Ni²⁺ cation was used instead of Mg²⁺ to promote more stable C3bBb complexes and provide for greater signal. Complexes were then washed and treated for 10, 20, or 30 min with either DAF’s functional CCPs 1–4 or buffer alone. C3bBb(Ni2⁺) complexes remaining were detected by ELISA using anti-human factor B goat polyclonal antibody. DAF sensitivity was determined for C3bBb(Ni2⁺) generated with each type A mutant and compared with that obtained with wild type C3bBb(Ni2⁺) as defined 100%.

The results are shown in Fig. 3 and Table I. Under the conditions used, most of the amino acid substitutions had no statistically significant effect on DAF-mediated decay acceleration. In contrast D254G, K265A/K266A (replacement of Lys-265 with A and Lys-266 with A), Q335A, Y338A, S339A, and M341A were markedly DAF-resistant, exhibiting 1–36% of the wild type activity (Table I and Fig. 4). As with DAF and CR1, major effects on factor H sensitivity were seen with N*260D and K265A/K266A (19–31% relative DAF sensitivity). Of the other substitutions that showed function in a factor D-dependent, divalent cation-linked glycans.

| Mutations        | DAF sensitivity | CR1 sensitivity | Factor H sensitivity |
|------------------|-----------------|-----------------|----------------------|
| Wild type        | 100%            | 100%            | 100%                 |
| D254G            | 9 ± 26% (0.007)  | 4 ± 26% (0.02)  | 48 ± 18% (0.008)     |
| N*260D           | 32 ± 19% (0.004)| 31 ± 17% (0.01)| 24 ± 23% (0.002)     |
| K265A/K266A      | 15 ± 31% (0.002)| 15 ± 30% (0.005)| 19 ± 31% (0.0002)    |
| K294A            | NS*             | 44 ± 18% (0.01) | NS                   |
| E301A            | 75 ± 9% (0.04)  | NS              | NS                   |
| E316A            | 71 ± 9% (0.02)  | NS              | NS                   |
| Q335A            | 20 ± 28% (0.02) | 22 ± 27% (0.03) | NS                   |
| Y338A            | 3 ± 31% (0.001)| 0 ± 34% (0.002)| 66 ± 22% (0.002)     |
| Y338F            | 18 ± 27% (0.008)| 20 ± 29% (0.004)| 61 ± 20% (0.003)    |
| Y338S            | 5 ± 25% (0.01)  | 24 ± 20% (0.08) | NS                   |
| S339A            | 32 ± 20% (0.007)| 50 ± 13% (0.03) | NS                   |
| M341A            | NS              | NS              | 75 ± 9% (0.009)      |
| W343A            | 55 ± 15% (0.02) | 56 ± 12% (0.04) | NS                   |
| E379A            | 52 ± 17% (0.01) | 50 ± 17% (0.02) | NS                   |
| D382A            | 36 ± 19% (0.003)| 41 ± 16% (0.001)| NS                   |
| D382N            | 54 ± 20% (0.02) | NS              | NS                   |
| E434A            | NS              | 65 ± 14% (0.01) | NS                   |
| D445A            | NS              | 71 ± 13% (0.01) | NS                   |

* NS, not statistically significant (p > 0.05).

Fig. 3. Effects of factor B mutations on DAF-mediated decay acceleration. C3bBb(Ni2⁺) assembled with wild type or mutant factor B protein were compared with respect to their sensitivity to DAF. The decay acceleration rate of wild type C3bBb(Ni2⁺) was defined as 100%.

TABLE I

Factor B mutations that affect decay acceleration

- Decay Acceleration Sites of the AP C3 Convertase

- Wild type 100% 100% 100%
- D254G 9 ± 26% (.007) 4 ± 26% (.02) 48 ± 18% (.008)
- N*260D 32 ± 19% (.004) 31 ± 17% (.01) 24 ± 23% (.002)
- K265A/K266A 15 ± 31% (.002) 15 ± 30% (.0005) 19 ± 31% (.0002)
- K294A NS* 44 ± 18% (.01) NS
- E301A 75 ± 9% (.04) NS NS
- E316A 71 ± 9% (.02) NS NS
- Q335A 20 ± 28% (.02) 22 ± 27% (.03) NS
- Y338A 3 ± 31% (.001) 0 ± 34% (.002) 66 ± 22% (.002)
- Y338F 18 ± 27% (.008) 20 ± 29% (.004) 61 ± 20% (.003)
- Y338S 5 ± 25% (.01) 24 ± 20% (.08) NS
- S339A 32 ± 20% (.007) 50 ± 13% (.03) NS
- M341A NS NS 75 ± 9% (.009)
- W343A 55 ± 15% (.02) 56 ± 12% (.04) NS
- E379A 52 ± 17% (.01) 50 ± 17% (.02) NS
- D382A 36 ± 19% (.003) 41 ± 16% (.001) NS
- D382N 54 ± 20% (.02) NS NS
- E434A NS 65 ± 14% (.01) NS
- D445A NS 71 ± 13% (.01) NS

- p values
- NS, not statistically significant (p > 0.05).
caused markedly increased resistance to DAF, Y338A and Y338F were statistically significant, but relatively minor compared with their effects on DAF and CR1 decay acceleration (relative to the wild type, Y338A conferred 66% factor H sensitivity but only 3% DAF sensitivity and 0% CR1 sensitivity). Moreover, C3bBb assembled with the D254G protein retained 48% factor H sensitivity, but only 9% DAF sensitivity and 4% CR1 sensitivity. No significant factor H effects were seen with Q335A, S339A, or D382A. Minor reductions in factor H sensitivity were seen with M341A, a substitution where no changes were detected in decay acceleration mediated by DAF or CR1.

Identification of Bb Structural Elements Involved in Decay Acceleration—As indicated, according to the model, the type A mutations made in this study are distributed over much of the domain surface (Fig. 2). In contrast, 12 of the 13 positions implicated in DAF/CR1 function were found in two discrete sites (Fig. 5); one site involves six amino acid positions and is defined by nine mutations (Q335A, Y338A, Y338F, Y338S, S339A, W343, E379A, D382A, and D382N). That site is centered on two adjacent α helices (α 4 and α 5). The four most critical positions in this region (Glu-335, Tyr-338, Ser-339, and Asp-382) constitute a discrete functional epitope (Fig. 6). The second site involves five amino acid positions and is defined by four mutations (D254G, N260D, K265A/K266A, E316A). That site consists of the α helix 1 and two adjacent loops and closely follows a portion of the previously reported C3b-binding region (see “Discussion”). Some mutations in the α 1 site dramatically affected factor H-mediated decay acceleration, while those in the α 4/5 site had, at most, very minor effects on factor H activity.

Assembly and Function of DAF-resistant C3bBb Complexes—The mutants that were most dramatically DAF- and CR1-resistant, seven carrying mutations in the α 4/5 site and three carrying mutations in the α 1 site, were compared with the wild type factor B protein for other biochemical differences. All ten
Decay Acceleration Sites of the AP C3 Convertase

Mutations in the C3 convertases are the central amplification enzymes of the complement cascade, and their proper regulation is essential to promote and facilitate elimination of foreign agents by effector cells while at the same time protecting self-tissues from complement-mediated destruction. A family of regulators, the RCA proteins, has evolved to serve this purpose, and these proteins are found both on cell surfaces and in the serum. In this regard, multiple RCA proteins can promote the dissociation (decay acceleration) of the C3 convertases. In this work we sought to map within Bb the biochemical interactions involved in this process.

Four of the human RCA proteins are capable of decay-acceleration: factor H can dissociate AP convertases, C4-binding protein (C4bp) can dissociate CP convertases, and CR1 and DAF can dissociate both AP and CP convertases. Of these four proteins, three can serve as cofactors for the factor I-mediated cleavage of C3b and/or C4b: factor II (C3b), C4bp (C4b), and CR1 (C3b and C4b). In the case of DAF the analysis of decay acceleration is simpler because it has no potentially confounding interactions providing for stable C3b or C4b binding or serving as a cofactor for factor I-mediated cleavage.

We began our search for DAF-Bb interaction sites by constructing a panel of factor B mutants, focusing on the type A domain because search for of the presence of C3b-binding elements in this region. Previous studies showed that when the isolated factor B type A domain is bound to C3b and the complex treated with trypsin, the type A peptide fragments that remain bound to C3b are from the apex of the domain, near the divergent cation-binding site (21) (Fig. 2A). These assignments were consistent with prior mutagenesis studies directed at the C3b-binding elements of factor B (16, 25).

Most of the mutants we prepared were single or double alanine substitutions that retained the capacity for C3bBb assembly and the function required for hemolytic activity. C3bBb complexes then were made with mutant or wild type factor B protein, and the DAF sensitivity of mutant complexes were compared with that of the wild type complexes. Several observations were made. 1) Mutations in 12 of 29 type A amino acid positions interfered with DAF-mediated regulation (Fig. 3 and Table I). 2) Two type A sites were involved in decay acceleration (Fig. 5). One site was centered around two adjacent α helices (α 4 and α 5) defined by six amino acid positions; a second site was located on the α 1 helix and two nearby loops defined by five amino acid positions in a region that includes C3b-binding elements. Two DAF-resistant α 1 mutations also markedly increased C3bBb stability (Table II). 3) The above two sites implicated in DAF-mediated decay acceleration are located on opposite sides of the type A domain (Fig. 5).

Three polar residues, Glu-335, Tyr-338, and Ser-339, and one acidic residue, Asp-382, appear to be most critical in the DAF/CR1 interaction. Together they form a contiguous patch on the surface of the type A domain model (Fig. 6). The tyrosine residue at position 338 is particularly relevant; alanine or serine substitution at this single position reduced decay acceleration to a few percent of wild type decay acceleration. Indeed, loss of the 4-hydroxyl group of the Tyr-338 side chain by phenylalanine substitution (Y338F) resulted in substantial loss in DAF sensitivity, suggesting an important role for hydrogen bonding at this point. Given the difference between Y338A and Y338F (Y338F is more active than Y338A), the Tyr-338 phenyl group also appears instrumental in the process. The side chain hydroxyl of Ser-339 is also of interest, since its removal (in S339A) also reduces DAF sensitivity. While it is impossible to determine whether the Tyr, Glu, and/or Ser side chains directly contact DAF, or instead are important structural elements of the α 4/5 contact region, tyrosine has been found to be a frequent “hot spot” of other protein interfaces (28, 29).

Each C3bBb complex was also examined for its behavior in the presence of CR1 or factor H. In general, factor B mutations that affected DAF-mediated decay acceleration similarly affected CR1-mediated decay acceleration (Fig. 4 and Table I). In contrast, fewer factor B mutations altered factor H decay acceleration activity. While the two DAF and CR1-resistant α 1 mutations also rendered C3bBb partially resistant to factor H, the most dramatic DAF and CR1-resistant α 4/5 mutants con-

### Table II

| Wild type | C3bBb(Ni²⁺) formation | C3bBb(Ni⁴⁺) decay | Hemolytic activity |
|-----------|----------------------|-------------------|-------------------|
|           | %                    |                   |                   |
| D254G     | 100                  | 100               | 100               |
| N²⁶⁰D     | 4902                 | 18                | 264               |
| K²⁶⁵A/K²⁶⁶A| 150                  | 43                | 91                |
| Q²⁵⁵Δ     | 94                   | 138               | 151               |
| Y³³⁸Δ     | 167                  | 72                | 377               |
| Y³³⁸F     | 179                  | 70                | 189               |
| Y³³⁸S     | 151                  | 54                | 212               |
| S³³⁹Δ     | 142                  | 88                | 191               |
| D³⁸²Δ     | 153                  | 92                | 232               |
| D³⁸²N     | 157                  | 99                | 367               |

**Fig. 6. Model of the factor B type A domain.** The α 4/5 amino acids most critical to decay acceleration are highlighted.
ferred, at most, only subtle changes in factor H sensitivity (Fig. 4 and Table I).

The α1 helix partially overlaps the C3b-binding region of the type A domain (Fig. 5). In contrast, the α4/5 site is somewhat removed from the C3b-binding region (Fig. 5). One simple interpretation of these relationships would be that DAF and CR1 interact with C3bBb at the α4/5 region and that mutations in the α4/5 region directly disrupt these interactions. In contrast, the α1 mutations would exert their effects directly on the C3b/Bb interface, obstructing changes in the interface that are at play during the dissociation of C3b from Bb, whether dissociation is spontaneous or accelerated by DAF, CR1, or factor H.

Although all three regulators were affected by some of the type A mutations, it appears that the mutation/response “spectra” of CR1 and DAF are quite similar, while that of factor H differs (Fig. 4 and Table I). This could imply that CR1 and DAF utilize similar biochemical mechanisms to promote decay acceleration. Both of these membrane-bound proteins require three CCPs for decay acceleration activity, CCP 2–4 of DAF and CCP 1–3 of CR1, with the two respective regions homologous by amino acid sequence and by intron/exon structure. The factor H protein is also encoded at the RCA cluster and composed of homologous CCPs, but it evolved as a serum protein, which could account for possible mechanistic differences.

Previous studies have shown that DAF CCPs 2–4 are involved in the decay acceleration of AP C3 convertase (8) and a positively charged region between CCP 2 and CCP 3 has been implicated in the process (12, 18). Given the importance of the factor B polar residues at the α4/5 region, the DAF α4/5 region can occur in more than one physical conformation (32). Thus, one possibility is that interaction of C3bBb with DAF or CR1 at the α4/5 site participates in promoting a type A conformation with relatively low affinity for C3b.

Acknowledgments—We thank Richard Hauhart for supplying the CR1-A protein and Madonna Bogacki for preparing the manuscript.

REFERENCES
1. Volanakis, J. E. (1998) in The Human Complement System in Health and Disease (Volanakis, J. E., and Frank, M. M., eds), 10th Ed., pp. 9–32, Marcel Dekker, Inc., New York.
2. Fearon, D. T., and Lockshen, R. M. (1996) Science 272, 50–54.
3. Carroll, M. C., and Prodeus, A. P. (1998) Curr. Opin. Immunol. 10, 36–40.
4. Nielsen, C. H., Rischer, E. M., and Leslie, R. G. Q. (2000) Immunology 100, 4–12.
5. Liszewski, M. K., and Atkinson, J. P. (1998) in The Human Complement System in Health and Disease (Volanakis, J. E., and Frank, M. M., eds), pp. 167–202, Marcel Dekker, Inc., New York.
6. Ahearn, J. M., and Rosengard, A. M. (1998) in The Human Complement System in Health and Disease (Volanakis, J. E., and Frank, M. M., eds), pp. 167–202, Marcel Dekker, Inc., New York.
7. Hourcade, D., and Atkinson, J. P. (1989) Prog. Immunol. 7, 171–177.
8. Brodbeck, W. G., Liu, D., Sperry, J., Mold, C., and Medof, M. E. (1996) J. Immunol. 156, 2528–2533.
9. Krych, M., Hauhart, R., and Atkinson, J. P. (1996) Mol. Immunol. 33, 439–446.
10. Kuhn, S., and Zipfel, P. F. (1996) Eur. J. Immunol. 26, 2383–2387.
11. Pangburn, M. K. (1996) J. Immunol. 156, 2216–2221.
12. Kuttner-Kondo, L. A., Mitchell, L., Hourcade, D. E., and Medof, M. E. (1996) Protein Eng. 9, 1143–1149.
13. Hourcade, D., Medof, M. E., Brodbeck, W., Wagner, L. M., and Oglesby, T. J. (1996) Mol. Immunol. 33, 57.
14. Hourcade, D. E., Wagner, L. M., and Oglesby, T. J. (1995) J. Biol. Chem. 270, 19716–19722.
15. Hourcade, D. E., Mitchell, L. M., and Oglesby, T. J. (1998) J. Biol. Chem. 273, 25996–26000.
16. Hourcade, D. E., Mitchell, L. M., and Oglesby, T. J. (1999) J. Immunol. 162, 2906–2911.
17. Brodbeck, W. G., Kuttner-Kondo, L., Mold, C., and Medof, M. E. (2000) Immunology 101, 104–111.
18. Kuttner-Kondo, L. A., Mitchell, L., Hourcade, D. E., and Medof, M. E. (2001) J. Immunol. 167, 2164–2171.
19. Krych, M., Clements, L., Howeshell, D., Hauhart, R., Hourcade, D., and Atkinson, J. P. (1994) J. Biol. Chem. 269, 13273–13278.
20. Lee, J. O., Rieu, P., Arnaout, M. A., and Liddington, R. (1996) Cell 80, 631–638.
21. Hinselwood, J., Spencer, G. R., Edwards, Y. J. K., and Perkins, S. J. (1999) J. Mol. Biol. 294, 587–599.
22. Sali, A., and Blundell, T. (1993) J. Mol. Biol. 234, 779–815.
23. Mele, J. E., Anderson, J. K., Davison, E. A., and Woods, D. E. (1984) J. Biol. Chem. 259, 3407–3412.
24. Lee, J. O., Bankston, L. A., Arnaout, M. A., and Liddington, R. C. (1995) Structure 3, 1333–1340.
25. Tuckwell, D. S., Xu, Y., Newham, P., Humphries, M. J., and Volanakis, J. E. (1997) Biochemistry 36, 6605–6613.
26. Michishita, M., Videm, V., and Arnaout, M. A. (1993) Cell 72, 857–867.
27. Krych-Goldberg, M., Hauhart, R. E., Bala Subramanian, V., Yurcisin, B. M., Crimmins, D. L., Hourcade, D. E., and Atkinson, J. P. (1999) J. Biol. Chem. 274, 31160–31168.
28. Hu, Z., Ma, B., Wolfsen, H., and Nussinov, R. (2000) Proteins 39, 331–342.
29. Bogan, A. A., and Thurn, K. S. (1998) J. Mol. Biol. 280, 1–9.
30. Emsley, J., Cruz, M., Handin, R., and Liddington, R. (1998) J. Biol. Chem. 273, 10407–10410.
31. Perkins, S. J., Hinselwood, J., Edwards, Y. J. K., and Jenkins, P. V. (1999) Biochem. Soc. Trans. 27, 815–821.
32. Hinselwood, J., and Perkins, S. J. (2000) J. Mol. Biol. 298, 135–147.
