

3.1. PREPARING RECOMBINANT PROTEINS FOR X-RAY CRYSTALLOGRAPHY

that genetic engineering be fully integrated into future crystallographic efforts, either directly within the crystallography laboratory or through close collaborations. There yet remain

formidable problems in protein structure and function that will require all the combined talents of the most skilled practitioners of these arcane arts.

References

- Abelson, J. N. & Simon, M. I. (1990). *Guide to protein purification. Methods Enzymol.* **182**, 1–894.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1995). *Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology*, 3rd ed. New York: Greene Publishing Associates and Wiley.
- Beggs, J. D. (1978). *Transformation of yeast by a replicating hybrid plasmid. Nature (London)*, **275**, 104–109.
- Bhandari, P. & Gowrishankar, J. (1997). *An Escherichia coli host strain useful for efficient overproduction of cloned gene products with NaCl as the inducer. J. Bacteriol.* **179**, 4403–4406.
- Biswas, E. E., Fricke, W. M., Chen, P. H. & Biswas, S. B. (1997). *Yeast DNA helicase A: cloning, expression, purification, and enzymatic characterization. Biochemistry*, **36**, 13277–13284.
- Bollag, D. M., Rozycki, M. D. & Edelman, S. J. (1996). *Protein methods*. New York: Wiley-Liss.
- Boyer, P. L. & Hughes, S. H. (1996). *Site-directed mutagenic analysis of viral polymerases and related proteins. Methods Enzymol.* **275**, 538–555.
- Brinkmann, U., Mattes, R. E. & Buckel, P. (1989). *High-level expression of recombinant genes in Escherichia coli is dependent on the availability of the dnaY gene product. Gene*, **85**, 109–114.
- Broach, J. R. (1983). *Construction of high copy number yeast vectors using 2 µm circle sequences. Methods Enzymol.* **101**, 307–325.
- Chong, S., Mersha, F. B., Comb, D. G., Scott, M. E., Landry, D., Vence, L. M., Perler, F. B., Benner, J., Kucera, R. B., Hirvonen, C. A., Pelletier, J. J., Paulus, H. & Xu, M. Q. (1997). *Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element. Gene*, **192**, 271–281.
- Chong, S., Shao, Y., Paulus, H., Benner, J., Perler, F. B. & Xu, M. Q. (1996). *Protein splicing involving the Saccharomyces cerevisiae VMA intein. The steps in the splicing pathway, side reactions leading to protein cleavage, and establishment of an in vitro splicing system. J. Biol. Chem.* **271**, 22159–22168.
- Cohen, S. L., Ferre-D'Amare, A. R., Burley, S. K. & Chait, B. T. (1995). *Probing the solution structure of the DNA-binding protein Max by a combination of proteolysis and mass spectrometry. Protein Sci.* **46**, 1088–1099.
- Cole, P. A. (1996). *Chaperone-assisted protein expression. Structure*, **4**, 239–242.
- Cregg, J. M., Vedick, T. S. & Raschke, W. C. (1993). *Recent advances in the expression of foreign genes in Pichia pastoris. Biotechnology*, **11**, 905–910.
- De Bernardes Clark, E. (1998). *Refolding of recombinant proteins. Curr. Opin. Biotechnol.* **9**, 157–163.
- De Boer, H. A. & Kastelein, R. A. (1986). *Biased codon usage: an exploration of its role in optimization of translation. In Maximizing gene expression*, edited by W. S. Reznikoff & L. Gold, pp. 225–285. Boston: Butterworths.
- Del Tito, B. J. Jr, Ward, J. M., Hodgson, J., Gershater, C. J. L., Edwards, H., Wysocki, L. A., Watson, F. A., Sathe, G. & Kane, J. F. (1995). *Effects of a minor isoleucyl tRNA on heterologous protein translation in Escherichia coli. J. Bacteriol.* **177**, 7086–7091.
- Enfors, S.-O. (1992). *Control of in vivo proteolysis in the production of recombinant proteins. Trends Biotechnol.* **10**, 310–315.
- Ernst, J. F. & Kawashima, E. (1988). *Variations in codon usage are not correlated with heterologous gene expression in Saccharomyces cerevisiae and Escherichia coli. J. Biotechnol.* **7**, 1–9.
- Ferré-D'Amaré, A. R. & Burley, S. K. (1994). *Use of dynamic light scattering to assess crystallizability of macromolecules and macromolecular assemblies. Structure*, **2**, 357–359.
- Fischer, B., Sumner, I. & Goodenough, P. (1993). *Isolation, renaturation, and formation of disulfide bonds of eukaryotic proteins expressed in Escherichia coli as inclusion bodies. Biotechnol. Bioeng.* **41**, 3–13.
- Georgiou, G. & Valax, P. (1996). *Expression of correctly folded proteins in Escherichia coli. Curr. Opin. Biotechnol.* **7**, 190–197.
- Gold, L., Pribnow, D., Schneider, T., Shinedling, S., Singer, B. S. & Stormo, G. (1981). *Translational initiation in prokaryotes. Annu. Rev. Microbiol.* **35**, 365–403.
- Goldman, E., Rosenberg, A. H., Zubay, G. & Studier, F. W. (1995). *Consecutive low-usage leucine codons block translation only when near the 5' end of a message in Escherichia coli. J. Mol. Biol.* **245**, 467–473.
- Gottesman, S. (1990). *Minimizing proteolysis in Escherichia coli: genetic solutions. Methods Enzymol.* **185**, 119–129.
- Grinna, L. S. & Tschopp, J. F. (1989). *Size distribution and general structural features of N-linked oligosaccharides from the methylotrophic yeast, Pichia pastoris. Yeast*, **5**, 107–115.
- Guise, A. D., West, S. M. & Chaudhuri, J. B. (1996). *Protein folding in vivo and renaturation of recombinant proteins from inclusion bodies. Mol. Biotechnol.* **6**, 53–64.
- Hendrickson, W. A., Horton, J. R. & LeMaster, D. M. (1990). *Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure. EMBO J.* **9**, 1665–1672.
- Hernan, R. A., Hui, H. L., Andracki, M. E., Noble, R. W., Sligar, S. G., Walder, J. A. & Walder, R. Y. (1992). *Human hemoglobin expression in Escherichia coli: importance of optimal codon usage. Biochemistry*, **31**, 8619–8628.
- Higgins, D. R. & Cregg, J. (1998). *Methods in molecular biology*, Vol. 103. *Pichia protocols*. Totowa: Humana Press.
- Hirel, P. H., Schmitter, M. J., Dessen, P., Fayat, G. & Blanquet, S. (1989). *Extent of N-terminal methionine excision from Escherichia coli proteins is governed by the side-chain length of the penultimate amino acid. Proc. Natl Acad. Sci. USA*, **86**, 8247–8251.
- Hockney, R. C. (1994). *Recent developments in heterologous protein production in Escherichia coli. Trends Biotechnol.* **12**, 456–463.
- Hofmann, A., Tai, M., Wong, W. & Glabe, C. G. (1995). *A sparse matrix screen to establish initial conditions for protein renaturation. Anal. Biochem.* **230**, 8–15.
- Hollenberg, C. P. & Gellissen, G. (1997). *Production of recombinant proteins by methylotrophic yeasts. Curr. Opin. Biotechnol.* **8**, 554–560.
- Hubbard, S. J. (1998). *The structural aspects of limited proteolysis of native proteins. Biochim. Biophys. Acta*, **1382**, 191–206.
- Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (1990). *PCR protocols: a guide to methods and applications*. San Diego: Academic Press.
- Jarvis, D. L., Kowar, Z. S. & Hollister, J. R. (1998). *Engineering N-glycosylation pathways in the baculovirus-insect cell system. Curr. Opin. Biotechnol.* **9**, 528–533.
- Jones, I. & Morikawa, Y. (1996). *Baculovirus vectors for expression in insect cells. Curr. Opin. Biotechnol.* **7**, 512–516.
- Kane, J. F. (1995). *Effects of rare codon clusters on high-level expression of heterologous proteins in Escherichia coli. Curr. Opin. Biotechnol.* **6**, 494–500.
- Kaufman, R. J. (1990). *Selection and coamplification of heterologous genes in mammalian cells. Methods Enzymol.* **185**, 537–566.
- Kim, R., Sandler, S. J., Goldman, S., Yokota, H., Clark, A. J. & Kim, S.-H. (1998). *Overexpression of archaeal proteins in Escherichia coli. Biotechnol. Lett.* **20**, 207–210.

3. TECHNIQUES OF MOLECULAR BIOLOGY

- Krueger, J. K., Kulke, M. H., Schutt, C. & Stock, J. (1989). *Protein inclusion body formation and purification*. *BioPharm*, March issue, 40–45.
- Kwong, P. D., Wyatt, R., Robinson, J., Sweet, R. W., Sodroski, J. & Hendrickson, W. A. (1998). *Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody*. *Nature (London)*, **393**, 648–659.
- LaVallie, E. R., DiBlasio, E. A., Kovacic, S., Grant, K. L., Schendel, P. F. & McCoy, J. M. (1993). *A thioredoxin gene fusion expression system that circumvents inclusion body formation in the E. coli cytoplasm*. *Biotechnology*, **11**, 187–193.
- LaVallie, E. R. & McCoy, J. M. (1995). *Gene fusion expression systems in Escherichia coli*. *Curr. Opin. Biotechnol.* **6**, 501–506.
- Lee, H. W., Joo, J.-H., Kang, S., Song, L.-S., Kwon, J.-B., Han, M. H. & Na, D. S. (1992). *Expression of human interleukin-2 from native and synthetic genes in E. coli: no correlation between major codon bias and high level expression*. *Biotechnol. Lett.* **14**, 653–658.
- Lu, A. & Miller, L. K. (1996). *Generation of recombinant baculoviruses by direct cloning*. *Biotechniques*, **21**, 63–68.
- McCarroll, L. & King, L. A. (1997). *Stable insect cell cultures for recombinant protein production*. *Curr. Opin. Biotechnol.* **8**, 590–594.
- McPherson, M. J., Hames, B. D. & Taylor, G. R. (1995). *PCR 2: a practical approach*. Oxford, New York: IRL Press at Oxford University Press.
- Makrides, S. C. (1996). *Strategies for achieving high-level expression of genes in Escherichia coli*. *Microbiol. Rev.* **60**, 512–538.
- Marston, F. A. (1986). *The purification of eukaryotic polypeptides synthesized in Escherichia coli*. *Biochem. J.* **240**, 1–12.
- Merrington, C. L., Bailey, M. J. & Possee, R. D. (1997). *Manipulation of baculovirus vectors*. *Mol. Biotechnol.* **8**, 283–297.
- Mitraki, A. & King, J. (1989). *Protein folding intermediates and inclusion body formation*. *Biotechnology*, **7**, 690–697.
- Mohsen, A.-W. A. & Vockley, J. (1995). *High-level expression of an altered cDNA encoding human isovaleryl-CoA dehydrogenase in Escherichia coli*. *Gene*, **160**, 263–267.
- Murby, M., Uhlén, M. & Ståhl, S. (1996). *Upstream strategies to minimize proteolytic degradation upon recombinant production in Escherichia coli*. *Protein Expr. Purif.* **7**, 129–136.
- Nilsson, B., Forsberg, G., Moks, T., Hartmanis, M. & Uhlén, M. (1992). *Fusion proteins in biotechnology and structural biology*. *Curr. Opin. Struct. Biol.* **2**, 569–575.
- O'Reilly, D. R., Miller, L. K. & Luckow, V. A. (1992). *Baculovirus expression vectors: A laboratory manual*. New York: W. H. Freeman & Co.
- Pfeifer, T. A. (1998). *Expression of heterologous proteins in stable insect culture*. *Curr. Opin. Biotechnol.* **9**, 518–521.
- Possee, R. D. (1997). *Baculoviruses as expression vectors*. *Curr. Opin. Biotechnol.* **7**, 569–572.
- Richardson, C. D. (1995). *Methods in molecular biology*, Vol. 39. *Baculovirus expression protocols*. Totowa: Humana Press.
- Richarme, G. & Caldas, T. D. (1997). *Chaperone properties of the bacterial periplasmic substrate-binding proteins*. *J. Biol. Chem.* **272**, 15607–15612.
- Ringquist, S., Shinedling, S., Barrick, D., Green, L., Binkley, J., Stormo, G. D. & Gold, L. (1992). *Translational initiation in Escherichia coli: sequences within the ribosome-binding site*. *Mol. Microbiol.* **6**, 1219–1229.
- Romanos, M. (1995). *Advances in the use of Pichia pastoris for high-level gene expression*. *Curr. Opin. Biotechnol.* **6**, 527–533.
- Romanos, M. A., Scorer, C. A. & Clare, J. J. (1992). *Foreign gene expression in yeast: a review*. *Yeast*, **8**, 423–488.
- Rossi, F. M. & Blau, H. M. (1998). *Recent advances in inducible expression systems*. *Curr. Opin. Biotechnol.* **9**, 451–456.
- Sachdev, D. & Chirgwin, J. M. (1998). *Solubility of proteins isolated from inclusion bodies is enhanced by fusion to maltose-binding protein or thioredoxin*. *Protein Expr. Purif.* **12**, 122–132.
- Saez, E., No, D., West, A. & Evans, R. M. (1997). *Inducible expression in mammalian cells and transgenic mice*. *Curr. Opin. Biotechnol.* **8**, 608–616.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular cloning: A laboratory manual*, 2nd ed. New York: Cold Spring Harbor Laboratory Press.
- Samuelsson, E., Moks, T., Nilsson, B. & Uhlén, M. (1994). *Enhanced in vitro refolding of insulin-like growth factor I using a solubilizing fusion partner*. *Biochemistry*, **33**, 4207–4211.
- Schein, C. H. & Noteborn, M. H. M. (1988). *Formation of soluble recombinant proteins in Escherichia coli is favored by lower growth temperature*. *Biotechnology*, **6**, 291–294.
- Schenk, P. M., Baumann, S., Mattes, R. & Steinbiss, H.-H. (1995). *Improved high-level expression system for eukaryotic genes in Escherichia coli using T7 RNA polymerase and rare Arg tRNAs*. *Biotechniques*, **19**, 196–198.
- Sclementi, C. R. & Calos, M. P. (1998). *Epstein-Barr virus vectors for gene expression and transfer*. *Curr. Opin. Biotechnol.* **9**, 476–479.
- Scopes, R. K. (1994). *Protein purification: principles and practice*. New York: Springer-Verlag.
- Shimotohno, K. & Temin, H. M. (1982). *Loss of intervening sequences in mouse α -globin DNA inserted in an infectious retrovirus vector*. *Nature (London)*, **299**, 265–268.
- Sorge, J. & Hughes, S. H. (1982). *Splicing of intervening sequences introduced into an infectious retroviral vector*. *J. Mol. Appl. Genet.* **1**, 547–559.
- Studier, F. W. & Moffatt, B. A. (1986). *Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes*. *J. Mol. Biol.* **189**, 113–130.
- Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990). *Use of T7 RNA polymerase to direct expression of cloned genes*. *Methods Enzymol.* **185**, 60–89.
- Tabor, S. & Richardson, C. C. (1985). *A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes*. *Proc. Natl Acad. Sci. USA*, **82**, 1074–1078.
- Tobias, J. W., Shrader, T. E., Rocap, G. & Varshavsky, A. (1991). *The N-end rule in bacteria*. *Science*, **254**, 1374–1377.
- Tsunasawa, S., Stewart, J. W. & Sherman, F. S. (1985). *Amino-terminal processing of mutant forms of yeast iso-1-cytochrome c. The specificities of methionine aminopeptidase and acetyltransferase*. *J. Biol. Chem.* **260**, 5382–5391.
- Unger, T. F. (1997). *Show me the money: prokaryotic expression vectors and purification systems*. *The Scientist*, **11**, 20–23.
- Wall, J. G. & Plückthun, A. (1995). *Effects of overexpressing folding modulators on the in vivo folding of heterologous proteins in Escherichia coli*. *Curr. Opin. Biotechnol.* **6**, 507–516.
- Waller, J.-P. (1963). *The NH₂-terminal residues of the proteins from cell-free extracts of E. coli*. *J. Mol. Biol.* **7**, 483–496.
- Werner, M. H., Clore, G. M., Gronenborn, A. M., Kondoh, A. & Fisher, R. J. (1994). *Refolding proteins by gel filtration chromatography*. *FEBS Lett.* **345**, 125–130.
- Wilkinson, D. L., Ma, N. T., Haught, C. & Harrison, R. G. A. (1995). *Purification by immobilized metal affinity chromatography of human atrial natriuretic peptide expressed in a novel thioredoxin fusion protein*. *Biotechnol. Prog.* **11**, 265–269.
- Yasukawa, T., Kanei-Ishii, C., Maekawa, T., Fujimoto, J., Yamamoto, T. & Ishii, S. (1995). *Increase of solubility of foreign proteins in Escherichia coli by coproduction of the bacterial thioredoxin*. *J. Biol. Chem.* **270**, 25328–25331.
- Yonemoto, W. M., McGlone, M. L., Slice, L. W. & Taylor, S. S. (1998). *Prokaryotic expression of catalytic subunit of adenosine cyclic monophosphate-dependent protein kinase*. In *Protein phosphorylation*, edited by B. M. Sefton & T. Hunter, pp. 419–434. San Diego: Academic Press.
- Zhang, S. P., Zubay, G. & Goldman, E. (1991). *Low usage codons in Escherichia coli, yeast, fruit fly and primates*. *Gene*, **105**, 61–72.