flanked by membranes of flat and parallel cisternae; the narrow interspace between the ribosomes and the membranes is evident[1]. The oblique section (fig. 3) shows the crystalline arrangement of the ribosomes in tetramers forming a P4 lattice, for which the optical diffraction pattern (fig. 3, insert) provides good evidence. At low magnification, the replicas obtained by the freeze-etching technique give some more information on the morphology of the ribosomal body cisternae, which appear to be flat, continuous and parallel. Figure 2 is a survey picture of a ribosomal body in which it is possible to observe that the IMPs are more numerous on the protoplasmic face (PF) of the membranes while on the endoplasmic face (EF) the particles are scanty and dispersed, as described in the RER. At higher magnification, in samples in which the fracture plane reveals a more extensive internal area of the membrane (fig. 4), two particle populations are recognizable on the PF: a more prominent and randomly dispersed one, and another formed by numerous flat IMPs arranged in a regular pattern. This pattern can often be visualized as parallel rows of particles with an interspace of about 600 Å. Occasionally, as in figure 4, a not well resolved 'square arrangement' of the IMPs can be seen, formed by intersecting perpendicular rows, which can be analysed by optical diffraction (fig. 4, insert).

The freeze-etching data, supported by the optical diffraction analysis, show a regular arrangement of the IMPs inside the membranes of the ribosomal bodies that can be correlated with the arrangement of the ribosomes in the crystalline sheets. In the three-dimensional model proposed by Unwin[4], each ribosome in a tetramer interacts with the membrane through a protrusion of the large subunit, and the four protrusions of a tetramer come together and touch the membrane at a site near the 4-fold axis.

Hence, the expected distance between two neighboring IMPs corresponding to ribosome binding sites should have the same value as the unit cell dimension (595Å) of the ribosome P4 crystal lattice[2]). As the interspace between two parallel rows of IMPs is about 600 Å, we can hypothesize that the ill-defined particles regularly arranged on the PF correspond to intrinsic proteins which bind to the ribosomes. The low resolution of these particles could depend on the absence of nascent polypeptides, since the ribosomes are functionally inactive[8].

It is to be noted that in the ovarian follicles of hibernating lizards, as well as in hypothermically treated lizard embryos, the ribosome crystals are always associated with membranes[9], unlike hypothermically treated chick embryos, where ribosome crystals are free in the cytoplasmic matrix[10,11]. This latter finding, together with the data in this report, suggests a direct role of the membranes in the process of ribosome crystallization in lizard tissues.

Acknowledgments. This work was carried out at the Electron Microscopy Center of the Faculty of Science, University of Naples, and was supported by an M.P.I. grant. The authors are endebted to Prof. W. H. Shreil of the International Institute of Genetics and Biophysics in Naples for suggestions throughout the present work and for the use of his optical bench.

1 Taddei, C., Exp. Cell Res. *102* (1972) 416.
2 Unwin, P. N. T., and Taddei, C., J. molec. Biol. *114* (1977) 491.
3 Unwin, P. N. T., J. molec. Biol. *132* (1979) 69.
4 Unwin, P. N. T., Nature *269* (1977) 118.
5 Kreibish, G., Freienstein, C. M., Pereyra, B. N., Ulrich, B. L., and Sabatini, D. D., J. Cell Biol. *27* (1978) 488.
6 Karnovsky, M. J., J. Cell Biol. *27* (1965) 137a.
7 DeRosier, D. J., and Klug, A., J. molec. Biol. *65* (1972) 469.
8 Taddei, C., Gambino, R., Metafora, S., and Monroy, A., Exp. Cell Res. *78* (1973) 159.
9 Taddei, C., Andreuccetti, P., and Bellini, L., J. submicrosc. Cytol. *13* (1981).
10 Byers, B., J. molec. Biol. *26* (1967) 155.
11 Maraldi, N. M., and Barbieri, M., J. submicrosc. Cytol. *1* (1969) 159.

0014-4754/87/080918-03$1.50 + 0.20/0

Fusion polypeptides in gene cloning: Potential problems due to conformational alterations at the junction

E. Querol and A. Parrilla

Instituto de Biología Fundamental V. Villar-Palasí and Departamento de Bioquímica, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona (Spain), 1 October 1986

Summary. Many eukaryotic genes are cloned in bacterial hosts as fusion polypeptides. Prediction of the secondary structures for some common prokaryotic fusion polypeptides shows that many junction sites correspond to important secondary structures. It is suggested that such structures could affect (hinder, etc.) the conformation or drive the folding of the neighboring eukaryotic counterparts. Thus the prokaryotic junction should be better performed in random coil regions, or short fusion prokaryotic polypeptides should be used.
Key words. Molecular cloning; fusion polypeptides; protein conformation; protein folding.

The fusion of eukaryotic to bacterial genes, thus producing a fused polypeptide, is commonly used to obtain cloned eukaryotic gene products in bacteria. Fused proteins are prepared for a better expression or secretion, and also for acquiring special antigenicity to facilitate screening procedures of the cloned products. Usually, the gene of interest is fused to the N-terminal portion of a bacterial or phage gene, and the hybrid gene is then transcribed from a strong bacterial promoter. These chimeric fusion proteins sometimes produce at levels of 10% or more of the total cellular protein, but there are also many examples of instability or lack of expression of the cloned protein, although its DNA sequence is in the correct reading frame. In other examples the cloning process yields polypeptide aggregates (termed inclusion bodies) other than native states. In fact, each protein poses a different problem[1].

An explanation for a number of the effects reported may be related to the protein folding pathway when this is driven or altered by the prokaryotic fused polypeptide. The present view of the protein folding process[2] assumes that the conformational information is dispersed through the polypeptide sequence, some positions being much more important than others in determining conformation. The folding process implies the formation of local reversible structures or nucleation centers: short stretches of secondary structures like α-helices of β-bends. Further stabilization comes from the establishment of interaction between neighboring structures. It is commonly thought that folding starts independently and more or less simultaneously in many regions of the polypeptide chain (autonomous folding domains). Thus, the prokaryotic fused polypeptide could affect the folding pathway by destabilizing a kinetically important intermediate in the

Vector	Fusion polypeptide	Secondary structures at the junction N-terminus*	C-terminus*+
pMC931	β-galactosidase	1–8 β-sheet	**8** β-sheet
pOP203-13	β-galactosidase	1–7 β-sheet	**7** β-sheet
M13mp2	β-galactosidase	1–7 β-sheet	139– **145** β-sheet
pβ-gal13C	β-galactosidase	1–7 β-sheet	997–**1005** α-helix
ptrpED5-1	tryptophan D	1–8 α-helix	71– **75** r. coil
pT465	tryptophan E	8–13 β-sheet	174– **190** β-sheet
pKT287	β-lactamase	1–23 β-sheet	**23** β-sheet

Some vectors and the corresponding secondary structures of both peptide ends. * Numbers indicate the size of the secondary structures involved. + Boldface indicates the residue number at the cutting site.

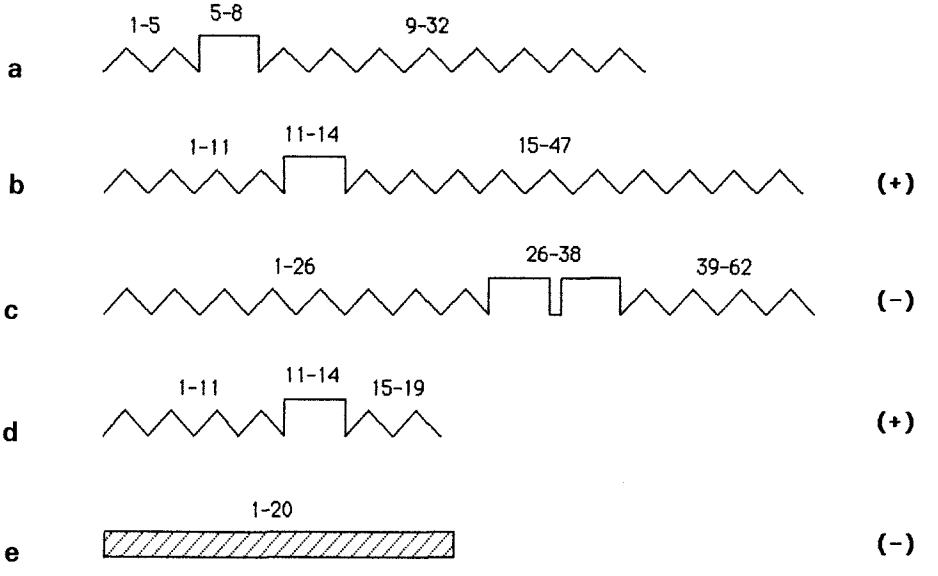
eukaryotic polypeptide. In addition, the eukaryotic N (or C) terminus may become buried, which can result in a non-native local protein conformation, thus yielding an insoluble or unstable form of the eukaryotic polypeptide. This insoluble state is a major consideration in the formulation of protocols for the purification of such protein products, since solubilization steps must be included, and only after reconstitution can the activity of the eukaryotic counterpart be detected; solubilization is carried out using denaturing agents, like guanidium chloride, followed by dialysis[3]. Thus, upon cleavage of the eukaryotic counterpart from the fused protein, the conformation of the eukaryotic polypeptide could remain in a non-native conformation attributable to a folding process driven by the prokaryotic counterpart.

The possibility that protein folding processes in prokaryotic cells follow different rules and pathways from those in eukaryotic cells has also to be taken into account. Their rules are less well-known than those for eukaryotes. Such a consideration could explain the observed tendency to form aggregates shown by eukaryotic proteins when cloned in prokaryotic cells[4].

For the above reasons, cutting and joining DNA fragments to build fusion proteins should be performed at sites not critical for the final native conformation of the eukaryotic protein or for intermediates of folding. The problem is that, at present, there is a major gap in the knowledge of which residues in the chain carry the folding information and which are the rules of the 'folding grammar', eukaryotic or prokaryotic. Nevertheless, we suggest that it should be wise to join the eukaryotic gene to a region of the prokaryotic counterpart predictable as a long stretch of random coil (e.g., 10 residues of random coil are probably unable to drive the folding of the neighboring eukaryotic region).

To check the type of secondary structures that some common cloning vectors present in their fusion polypeptides[5], we have predicted their secondary structures – according to Chou and Fasman[6] – and their hydropathic profiles – according to Kyte and Doolittle[7] – by means of a computer program previously described[8]. The table summarizes the secondary structure involved in different reported junction sites for some vectors. As can be seen in the table, many fusion polypeptides are built by joining the eukaryotic gene to a 'pre-existent' well-defined secondary structure (helices or sheets). Some vectors fuse the eukaryotic polypeptide as the N-terminus of the prokaryotic counterpart, but according to the present view of the folding process as multi-nucleation centers it is also plausible that the prokaryotic C-terminus could drive regions of the eukaryotic N-terminus. The multi-nucleation center hypothesis also gives a possible explanation for the fact that in spite of the above considerations, many cloned genes have been successfully expressed as fusion proteins: the dispersion of conformational information through the sequence together with the modular structure (domain) of proteins would permit many eukaryotic fused polypeptides to follow folding pathways independent of the prokaryotic counterparts.

A method to test our hypothesis would be to analyze enzymes cloned as different fusion proteins and assay their activity in each case. However, few proteins have been cloned twice or more, via different fusion polypeptides, and there are even fewer proteins whose activity has been measured and reported. Most of the reported cloned genes are those detected by immunological methods. Nevertheless, a positive immunological response can give rise to different polypeptide conformation due to continuous antigenic determinants. Another way to test the above hypothesis is to search for differences in the production of immunologically active surface antigens from different plasmidic constructs. For example, MacKay et al.[9] have reported the cloning of the hepatitis B surface antigen in *E. coli*. They found that some recon-



N-terminus secondary structures predicted for: a) native HBsAg. b–e) different HBsAg constructs[9].

Symbols: +/– indicate high/low levels of immunogenicity. beta sheet = ⩘ ; alpha helix = ▨ ; beta turn = ⊓.

structed plasmids yielded recombinant polypeptides presenting very different immunogenicity. We have performed the Chou and Fasman prediction of some of their most representative (high or low immunogenicity) constructs (those named 1, 3, 4 and 10 by these authors). The figure shows the prediction of the fusion polypeptide N-terminus compared with the structure prediction of the HBsAg protein. The corresponding immunogenicities reported by these authors are also indicated. As can be seen in the figure, the long stretch of β-sheet in construct 'C' alters the fusion protein and results in low immunogenic response, most likely by hindrance caused on the next HBsAg peptide. Construct 'E' shows that the long N-terminal stretch of α-helix in the fusion polypeptide continues into the HBsAg peptide, which also has low immunogenicity.

Another example is the cloning of Foot and Mouth viral antigenic peptide (142–160) as the N-terminus of a fusion polypeptide with residues 9-1023 of β-galactosidase[10]. In this case, the modification would occur in the β-galactosidase peptide, whose junction region changes from a β-sheet to a turn (prediction not shown).

The above considerations can also be extended to protein engineering. At present, it is possible to envisage the design of new proteins via fusion of two or more polypeptides, or domains. In all cases in which new combinations of DNA sequences corresponding to pre-existent protein structures are produced, problems like those discussed above may appear.

Acknowledgment. This work was financially supported by CAICYT (AG 28–82, Ministerio Educación y Ciencia) and FIS (86–569, Ministerio de Sanidad).

1 Harris, T.J.R., Expression of eukaryotic genes in *E. coli*, in: Genetic Engineering, vol. 3, pp. 128–185. Ed. R. Williamson. Academic Press, New York 1983.
2 King, J., Biotechnology *4* (1986) 297.
3 Goedel, D.V., Kleid, D.G., Bolivar, F., Heynecker, H.C., Yansura, D.G., Crea, R., Hirose, T., Kraszewski, A., Itakura, K., and Riggs, A.D., Proc. natn. Acad. Sci. USA *76* (1979) 106.
4 Williams, D.C., Van Frank, R.M., Muth, W.L., and Bennet, J.P., Science *215* (1982) 687.
5 Cloning Vectors, A Laboratory Manual, Eds P.H. Pouwels, B.E. Enger-Valk and W.J. Brammar. Elsevier, Amsterdam 1985.
6 Chou, P.Y., and Fasman, G.D., Adv. Enzymol. *47* (1978) 45.
7 Kyte, J., and Doolittle, R.F., J. molec. Biol. *157* (1982) 105.
8 Parrilla, A., Domènech, A., and Querol, E., Comp. appl. Biosc. *2* (1986) 211.
9 MacKay, P., Pasek, M., Magazin, M., Kovacic, R.T., Allet, B., Stahl, S., Gilbert, W., Schaller, H., Bruce, S.A., and Murray, K., Proc. natn. Acad. Sci. USA *78* (1981) 4510.
10 Winther, M.D., Allen, G., Bomford, R.H., and Brown, F., J. Immun. *136* (1986) 1835.

0014-4754/87/080920-03$1.50 + 0.20/0

Glischropus tylopus, the first known old-world bat with an X-autosome translocation

M. Volleth[1,2] and H.S. Yong*

*Dept. of Human Genetics, Schwabachanlage 10, D-8520 Erlangen (Federal Republic of Germany) and *Dept. of Zoology, University of Malaya, 59100 Kuala Lumpur (Malaysia), 23 January 1987*

Summary. Glischropus tylopus shows an X-autosome translocation in which a small acrocentric chromosome has been transferred to the X. The diploid number is 30 for the females and 31 for the males. RBG banding shows that in the late replicating X of the female only the original X replicates late, the autosomal part replicates early, showing the same pattern as the corresponding autosomal 'Y_2' of the male. In the X chromosome, a heterochromatic band separates the autosomal from the gonosomal sequences.

Key words. Vespertilionidae; X-autosome translocation; late replicating X; spreading effect.

The genus *Glischropus* (family Vespertilionidae), which is considered to be an offshoot of the widespread genus *Pipistrellus*[3], consists of only two species, i.e. *G. javanicus*, which occurs in Java and has not been karyologically investigated, and *G. tylopus*, occurring in the other parts of South East Asia, which will be treated here.

Glischropus tylopus shows as an interesting morphological specialization white or pink colored pads on thumb and feet, which are considered to be useful for climbing in hollow tubes, e.g. bamboo[4], as is proven for *Tylonycteris*[5] bats (which show, however, differently shaped pads).

Five specimens of *Glischropus tylopus* were karyologically examined, all captured near the University of Malaya Field Study Centre (3°20'N, 101°45'E), Ulu Gombak, Selangor, Malaysia in 1984. Conventionally stained preparations from lymphocyte culture were obtained from three animals (2 males, one female). Fibroblast cultures of heart and lung tissue were established from one male and one female. These slides were used for duplicate staining (AgNOR[6] or CBG[7] banding after quinacrine[8]) or GTG[9] banding. RBG bands[10] were obtained after a 10-h block of thymidine followed by an 8-h BrdU treatment[11].

Results. The karyotype consists of 30 chromosomes in the females and 31 chromosomes in the males. There are 8 large metacentric to submetacentric, two small subtelocentric and 4 acrocentric autosomal pairs. The X chromosome is a large submetacentric one, the two unmatching chromosomes of the males are small acrocentric chromosomes of differing size. After G banding (fig. 1) each pair can be unequivocally identified. The smaller of the two unmatching acrocentric chromosomes of the male shows both the same size and the same faint staining as the short arm of the X chromosome (fig. 1). Therefore it will be regarded as Y_2 which is of autosomal origin. After NOR staining one submetacentric pair shows an NOR in the short arm close to the centromere (see arrow in fig. 1). The centromeres are only faintly stained after C banding, but a broad band of constitutive heterochromatin can be seen in the short arm of the X close to the centromere, thus separating the gonosomal from the autosomal genes (fig. 3). Additionally, the larger of the two unmatching chromosomes of the male is, except for a very narrow distal region, completely heterochromatic. This chromosome is therefore regarded as Y_1, representing the original Y chromosome.

This assignment is confirmed by the results of RBG banding after BrdU incorporation (fig. 2). The X chromosome of the male and the early replicating one in the female show several early replicating bands in the long arm and a broad early