CARYOLOGIA Vol. 59, no. 4: 354-357, 2006

Two phenotypes with a single genotype: the case of butterfly

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Abstract — To better understanding the plastic nature of the gene, we analysed single genotypes that determined two alternative phenotypes (for examples by the sex determination of reptiles and vernalization of plants). Here we used the model of butterfly eye spots to highlight how different embryonic developmental genetic pathways can be the basis of evolution not by darwinian selection.. Further, we provided evidence that specific properties of molecules are the basis of the mechanism by which a gene "detects" its environment and consequently acts.

Key words: butterfly eyes spots, b-HLH protein, gene-environment interaction.

INTRODUCTION

The gene action is too often considered in a deterministic manner. Because of the undervaluation of the weight of gene and environment interactions, for example in humans, to the gene it is attributed the function of the exclusive behaviour and disease determinant. The gene action is, instead much more plastic: here we examined the function of a gene that, because of its interaction with the environment, is capable of producing two alternative phenotypes.

The intriguing case is that of a butterfly (*Bicyclus anyana*) (Fig. 1), living in Mali and that shows different phenotypes in wing pattern in response to temperature changes (Galant *et al*. 1998). At 17°C, in fact the eyespots in the wings are not present whereas they are greatly visible at 27°C. Only one gene is involved in the production of wing eyespots. The product of this gene is a b-HLH-protein responsible for both the formation of the wing scales and the overlying pattern. How senses the b-HLH-protein the changes in the temperature and reform oneself to the different function? The amino-acid sequence of this protein (Fig: 2), predicted by nucleotide sequence, is similar to the protein family containing the b-HLH (basic-helix-loop-helix) motif (Galant *et al*. 1998). All of the b-HLH proteins act as transcription factor when are in dimeric conformation. As dimer these protein are capable of binding DNA, whereas as monomer they falls to bind DNA sequences (Gigliani *et al.* 1996).

This relevant property suggests that the predicted b-HLH-protein detected in the butterfly fluctuates between dimeric and monomeric conformation according to temperature and then represent the tester by which the HLH encoding gene decides between the two alternative phenotypes.

To verify this hypothesis, we compared the butterfly HLH amino acid sequence with the well-known b-HLH sequences and tested whether amino acid substitutions are such as to invalidate the typical tridimensional structure of the butterfly b-HLH protein.

When the butterfly b-HLH sequence is compared with the sequences of the b-HLH domains of other proteins (Fig. 3), we observed that the amino acid differences in the butterfly b-HLH are frequently present also in the other b-HLH motives. Therefore, the b-HLH of the butterfly should maintain the typical b-HLH conformation. Furthermore, taking MyoD protein as model, we substituted *in silico* (DeepView-The Swiss-Pdp Viewer program. http:/www.expasy-.org.spdbv) the amino acids of MyoD b-HLH with the amino acids that differ in the butterfly b-HLH sequence. The comparison of the tridimentional structure of MyoD (showed in Fig. 4) with the tridimentional model of the butterfly b-HLH in Fig. 5, does not reveal conformational changes such that the amino acids substitutions may give rise to a loss of typical HLH conformation in the butterfly b-HLH protein. Amino acids of both MyoD HLH and butterfly HLH, lay on the same

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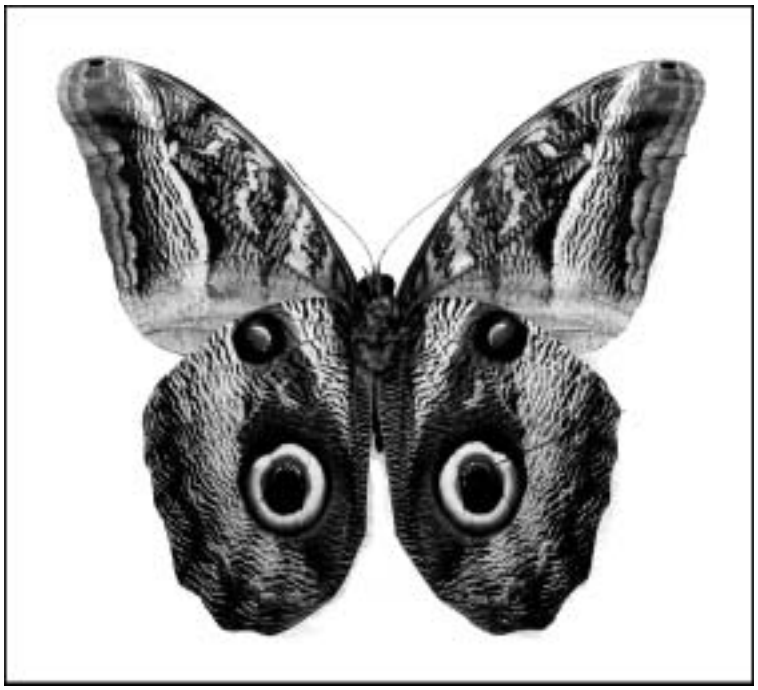

Fig. 1 — The butterfly *Bicyclus anyana*.

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1   ATGTTACAAGAAATACAATTAGTTCAAGTTCAGACCAACTACGTGGTCGT  50
     M  L  Q  E  I  Q  L  V  Q  V  Q  T  N  Y  V  V  V
51  CTCCTCTGGCTATCCCTCCGCGACTGTTACAAAACTTTCGTTGGAAAAAA 100
      S  S  G  Y  P  S  A  T  V  T  K  L  S  L  E  K
101 GGATTGTACCGATTGCACCGCTCCAGAGAAAAATTATGTCACTCACGAC 150
    R  I  V  P  I  A  P  A  P  E  K  N  Y  V  T  H  D
151 ACGCCGCCCAACCTACAGTACAGAAAGAAGGTACATTTTAGAACGAACCC 200
     T  P  P  N  L  Q  Y  R  K  K  V  H  F  R  T  N  P
201 CTACACTGGACCGCAGGCTGCCTCGATTGCGAGACGAAATGCGCGTGAAC 250
      Y  T  G  P  Q  A  A  S  I  A  R  R  N  A  R  E
251 GTAATCGCGTGAAACAAGTAAACGATGGATTCAATGCACTTCGCCGTCAT 300
    R  N  R  V  K  Q  V  N  D  G  F  N  A  L  R  R  H
301 CTGCCAGCTTCCGTAGTGGCCGCTTTATCTGGCGGCGCCAGACGGGGATC 350
     L  P  A  S  V  V  A  A  L  S  G  G  A  R  R  G  S
351 CGGAAAGAAACTCAGTAAAGTTGATACGTTACGAATGGTCGTTGAATATA 400
      G  K  K  L  S  K  V  D  T  L  R  M  V  V  E  Y
401 TCAGATACTTGCAACAATTACTTGATGAGAGTGATGCTGCCTTGGGTATT 450
     I  R  Y  L  Q  Q  L  L  D  E  S  D  A  A  L  G  I
451 ACACGTGATCAAGAAAATAGAGAAAATATTCCTAATAGCAATTCACAAAG 500
     T  R  D  Q  E  N  R  E  N  I  P  N  S  N  S  Q  S
501 TATGAATTCTATAGCGGATATGGATGACGGTTTCTTCTACGGAAGCAGTT 550
      M  N  S  I  A  D  M  D  D  G  F  F  Y  G  S  S
551 CGCCGTGTTCTGAAAAGGCGGATTCCCCTGCACCTTCGGAGTGTTCGTCT 600
    S  P  C  S  E  K  A  D  S  P  A  P  S  E  C  S  S
601 GGTGTGTCGTCTGCGTACTCAGCGGTTGACCGATACGAAGTGTCAACGCA 650
     G  V  S  S  A  Y  S  A  V  D  R  Y  E  V  S  T  Q
651 GCAACCCCTCGGGCCCATGGATGAGGACGAGCTGCTGGACGTGATATCGT 700
      Q  P  L  G  P  M  D  E  D  E  L  L  D  V  I  S
701 GGTGGCAGCAGAAATAG
    W  W  Q  Q  K  *
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Fig. 2 — Nucleotide sequence and predicted amino acids sequence of butterfly protein. The basic- helix-loop-helix motif is boxed.

Fig. 3 — Alignment of sequences of b-HLH domains. In bold are indicated the amino acid residues that differ in the butterfly b-HLH motif.

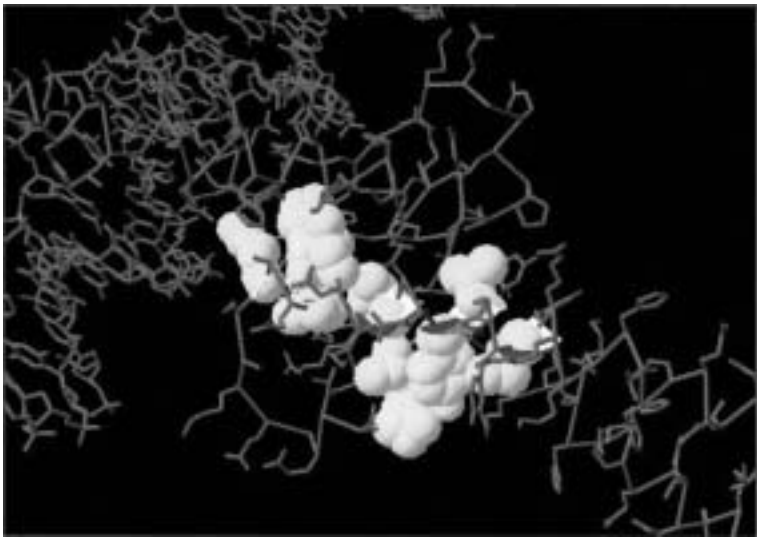

Fig. 4 — Tridimentional structure of MyoD protein. In white are the amino acid residues of b-HLH domain involved in hydrophobic bonds.

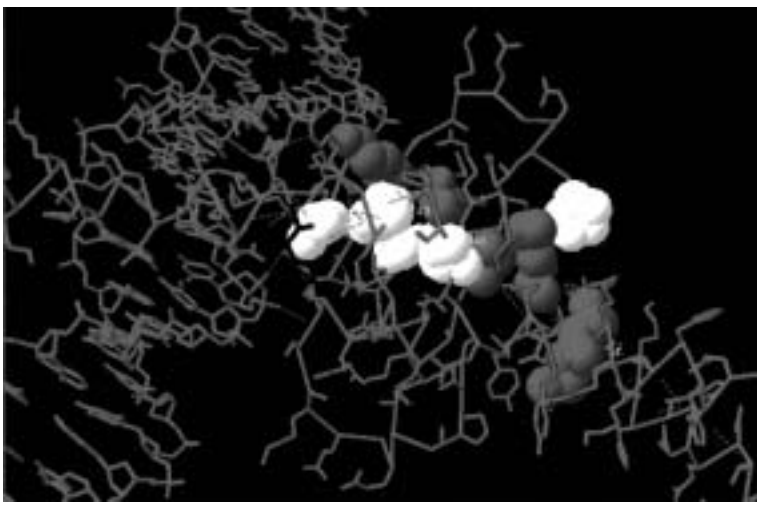

Fig. 5 — Tridimensional structure of MyoD protein with butterfly amino acids substitution (in grey).

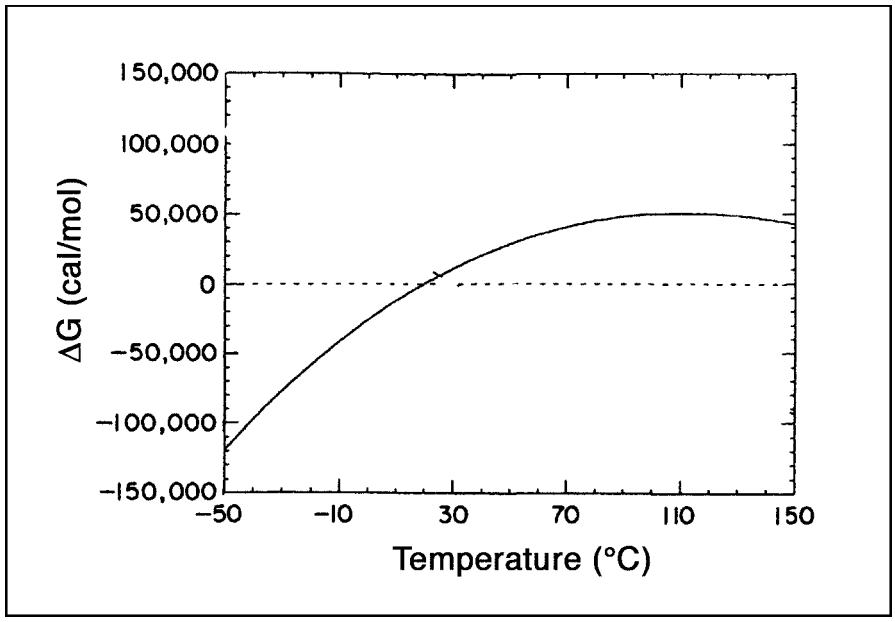

Fig. 6 — Graphic showing the ΔG variation of hydrophobic bond formation at different temperature.

position in the tridimentional structure producing the interaction between hydrophobic amino acids that are essentially the same in both cases.

This result strongly suggests that effectively the butterfly protein is a b-HLH protein not only for the similarity in the amino acid sequence, but also because the various substitutions maintain the typical tridmensional HLH structure.

Since the butterfly HLH motif exhibits the same conformation of the other HLH known, we tested whether the properties of this protein account for two different quaternary structures at different temperature. The butterfly HLH monomer or dimer formation depends on the amino acids capable of forming hydrophobic bonds. The hydrophobic bond is more stable at higher temperature and less at lower temperature. As shown by the graphic in Fig. 6, a temperature shift from 17°C to 27°C favours at 27°C dimer formation, whereas at 17°C favours monomer formation.

In conclusion, the structure and the properties of the butterfly HLH justify the choice of this protein as molecule that senses the environment and consequently acts in such a way that the butterfly b-HLH encoding gene produces two different phenotypes depending on the environment.

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