Backward bicoid?

From Steven D Hanes and Roger Brent: We wish to point out some apparent errors in the paper by Masashi Suzuki, in the 15 April issue of *Structure* [1]. This paper discusses chemical and stereochemical 'rules' for protein–DNA interaction. The author suggests that these rules might be useful for assigning amino acid–base pair contacts to DNA-binding proteins of the probe-helix type, in which he includes homeodomain proteins.

Suzuki stated (p. 324) that "The N to C direction of the bicoid protein on the DNA seems to be wrongly assigned in the previous work". We did in fact propose the alignment of the bicoid recognition-helix on the DNA [2]. We argued, based on genetic experiments, that the bicoid recognition helix is inserted into the major groove of DNA with its amino terminus towards the 3′ end of the bicoid site (5′-TCTAATCCC) and its carboxyl terminus towards the 5′ end of the site. This alignment is exactly what Suzuki now proposes based on his stereochemical rules. In Fig. 5i of his article, Suzuki wrongly depicts our published alignment. In fact, the alignment we proposed (Fig. 3 in [2]) is identical to Suzuki's corrected alignment in Fig. 5h. Not only do we agree with the alignment predicted by Suzuki, but our previously published work provides experimental support for such a model.

Second, in Figs 5i and 5h, Suzuki indicates base-specific contacts by Lys9 of the bicoid recognition helix to two different positions in the bicoid site (TCTAATCCC). Our results [2] established that there is only a single base-specific contact by Lys9 (TCTAATCCC) and that contacts to the other position were not strictly base-specific. Finally, the finding that bicoid's binding specificity depends on Lys9 of its recognition helix (position 4 in Suzuki's nomenclature) was incorrectly attributed and should be Hanes and Brent (1989) [3].

We think it is possible that the confused alignment in the Suzuki paper might have resulted from the fact that the engrailed site, to which the bicoid site was compared, is nearly palindromic. It appears that the TAAT core of the bicoid site may have been misaligned with a TAAT on the opposite strand in the engrailed site. Below, we show the relevant alignment of the bicoid site with those of engrailed, antennapedia, fushi tarazu and Matα2 based on our genetic studies [2], and the structural studies of Kissinger *et al.* [4], Otting *et al.* [5] and Wolberger *et al.* [6] and the genetic and biochemical studies of Schier and Gehring [7], and Percival-Smith *et al.* [8].

bicoid	5′-TC**TAAT**CCC-3′
engrailed	5′-TG**TAAT**TAC-3′
antennapedia	5′-TC**TAAT**GGC-3′
fushi tarazu	5′-TG**TAAT**TGC-3′
Matα2	5′-AA**TTAC**ATG-3′

The sites are aligned based on DNA contacts made by amino acids at equivalent positions in each protein. For example, in each case, amino acid 50 of the homeodomain (residue 9 in the recognition helix) contacts base pairs (underlined) that are located 3′ to a central TAAT core sequence. For homeodomains in which the conserved Asn51 contact has been identified, it is to the third position of the TAAT core sequence (TTAC for the divergent Matα2 protein). For each homeodomain protein, the recognition helix is aligned with its amino terminus towards the 3′ end of the site and its carboxyl terminus towards the 5′ end of the site.

We expect that together with structural and biochemical studies, careful genetic analysis will continue to illuminate important structural issues and provide a useful and independent means of testing chemical and stereochemical rules for protein–DNA recognition such as those proposed by Suzuki.

Masashi Suzuki replies: Concerning the N to C orientation of the bicoid protein, my original statement is incorrect and I apologise to Drs Hanes and Brent for this error. It arose from a misunderstanding of the discussion of the NMR structure of the DNA–homeodomain complex. However, this does not affect any of the DNA recognition rules described in my paper, as acknowledged by Hanes and Brent.

Whether the lysine residue binds to only one base or two, the conclusion reached by Treisman *et al.* [9] is different from that of Hanes and Brent [2]. Treisman *et al.* state "This suggests that the homeobox encodes a domain recognizing a TAATNN sequence, the identity of residue 9 determining the specificity for recognition of the two bases NN." In any case, this detail does not significantly affect the stereochemical rules as proposed in [1].

The sequence alignment shown above is totally consistent with my stereochemical charts (Matα2, Fig. 4i; engrailed, Figs. 4h and 5g; bicoid, Fig. 5h).

References

1. Suzuki, M. (1994). A framework for the DNA–protein recognition code of the probe helix in transcription factors: the chemical and stereochemical rules. *Structure* **2**, 317–326.
2. Hanes, S.D. & Brent, R. (1991). A genetic model for interaction of the homeodomain recognition helix with DNA. *Science* **251**, 426–430.
3. Hanes, S.D. & Brent, R. (1989). DNA specificity of the Bicoid activator protein is determined by homeodomain recognition helix residue 9. *Cell* **57**, 1275–1283.
4. Kissinger, C.R., Liu, B., Martin-Blanco, E., Kornberg, T.B. & Pabo, C.O. (1990). Crystal structure of an engrailed homeodomain–DNA complex at 2.8 Å resolution: a framework for understanding homeodomain–DNA interactions. *Cell* **63**, 579–590.
5. Otting, G. *et al.*, & Wüthrich, K. (1990). Protein–DNA contacts in the structure of a homeodomain–DNA complex determined by nuclear magnetic resonance in solution. *EMBO J.* **9**, 3085–3092.
6. Wolberger, C., Vershon, A.K., Liu, B., Johnson, A.D. & Pabo, C.O. (1991). Crystal structures of a Matα2 homeodomain–operator complex suggests a general model for homeodomain–DNA interactions. *Cell* **67**, 517–528.
7. Schier, A.F. & Gehring, W.J. (1992). Direct homeodomain–DNA interaction in the autoregulation of the *fushi tarazu* gene. *Nature* **356**, 804–807.
8. Percival-Smith, A., Müller, M., Affolter, M. & Gehring, W.J. (1990). The interaction with DNA of wild-type and mutant fushi tarazu homeodomains. *EMBO J.* **9**, 3967–3974.
9. Treisman, J., Harris, E., Wilson, D. & Desplan, C. (1992). The homeodomain: a new face for the helix-turn-helix? *Bioessays* **14**, 145–150.

Steven D Hanes, Axelrod Institute, Wadsworth Center, New York State Department of Health, Albany NY 12208, USA and Roger Brent, Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston MA 02114, USA.
Masashi Suzuki, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK.