tified; each consisted of an in-frame exchange of 9 to 48 base pairs (bp) from the C1H4 gene segment into unrelated rearrangements of V186.2. In the sample from $B220^{lo}$ GC cells, only a single V_H V23 rearrangement encoded the YYYGS motif in CDR3, and three rearrangements, all V186.2, were not in-frame. Two of these nonproductive (nP) rearrangements resulted from incomplete codons at the V-D-J junction, whereas the third contained a complex 215-bp insertion of DNA from the V186.2 and V_H 165.1 gene segments. Two hybrid sequences were present: the 215-bp insert described earlier and an in-frame exchange of 30 bp from the C1H4 exon into a productive V186.2 rearrangement.

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TECHNICAL COMMENT

BMP Expression in Duck Interdigital Webbing: A Reanalysis

In 1996, two of us (H.Z. and L.N.) reported that expression of a dominant-negative form of BMP receptor (*dnBMPR-IB*) in the embryonic chick hindlimb inhibited interdigital apoptosis and led to webbing of the digits (*1*) (BMPs are signaling molecules of the transforming growth factor–β superfamily). The importance of BMP signaling in regulating interdigital cell death has recently been confirmed by the use of an activated *BMPR-IB* retrovirus (*2*) and by application of BMP protein (*3*, *4*).

It was also stated in this report (*1*) that *BMP2*, *4*, and *7* RNA expression was not detected in the duck interdigit. This result implied that the webbing in the hindlimb of ducks is a consequence of the absence of BMP expression in the duck embryo. After publication of the report (*1*), to explore this issue further, subsequent in situ hybridizations were carried out with the use of a modification (*5*) of an existing whole mount, in situ protocol. Results from our two different laboratories now indicate that *BMP2*, *4*, and *7* are in fact expressed in the duck interdigit in a pattern similar to that of the chick interdigit (Fig. 1). The in situ protocol we used, in contrast with the protocol followed in the original study (*1*), included use of (i) a higher proteinase K concentration (30 to 70 μg/ml rather than 5 μg/ml), (ii) BCIP/NBT (*6*) as a color detection substrate rather than Boehringer-Mannheim purple AP substrate, and (iii) a TWEEN-20 concentration of 1% rather than 0.1% during the color substrate reaction. In combination, these modifications resulted in a greater sensitivity in detecting interdigital expression of *BMP2*, *4*, and *7* in late-stage embryonic limbs. This result has been confirmed for *BMP7* by nonradioactive in situ hybridization to frozen tissue sections (*7*). Therefore, we (H.Z. and L.N.) must withdraw the earlier finding that the duck interdigit lacks BMP expression and regret any inconvenience the earlier conclusions may have caused.

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5. Whole mount in situ hybridizations were performed as described by R. D. Riddle *et al.* [*Cell* **75**, 1401 (1993)], except that the temperature of all hybridizations and post-hybridization washes was 60°C and the proteinase K (PK) conditions were varied. Standard 1× PK is nominally 10μg/ml for 15 min at room temperature. However, we have noted batch variation in the specific activity of PK, and the conditions must therefore be titrated for each batch. Typically, 1× PK treatment results in the removal of most or all of the signal from s22 limb bud AERs, and strong mesenchymal signal for genes such as *BMP2* or *Sonic hedgehog*. Maximal AER staining is usually seen around 1/4× PK. Older embryos often require substantially more PK treatment to reveal strong mesenchymal signals (up to 10× for 9-day-old embryonic limbs). In the experiments described here, PK conditions were independently optimized for visualization of interdigital BMP expression in both chick and duck limb buds. Following PK digestion, stage-matched chick and duck embryos were combined into a single vial and treated together for the remainder of the protocol. NBT/BCIP reactions were performed at room temperature for varying amounts of time (typically 1.5 to 3 hours), until a strong interdigital signal was observed. All of the probes were derived from the chick BMP genes, as described in Zou and Niswander (*1*).
6. BCIP, 5-bromo-4-chloro-3-indolyl phosphate; NBT, nitro blue tetrazolium; both available from Sigma.
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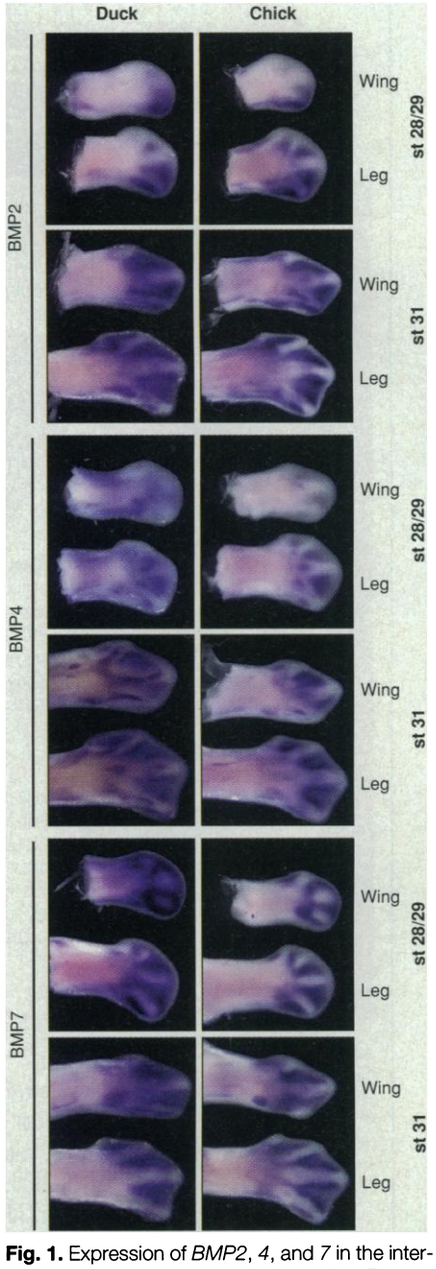

Fig. 1. Expression of *BMP2*, *4*, and *7* in the interdigital regions of chick and duck limbs. Expression of each gene was detected by whole mount in situ hybridization to stage 28/29 and to stage 31 chick (*8*) and stage-equivalent duck embryos, as indicated. In each case, wing and leg buds were removed from the embryos after completion of the whole mount procedure and photographed from a dorsal view.