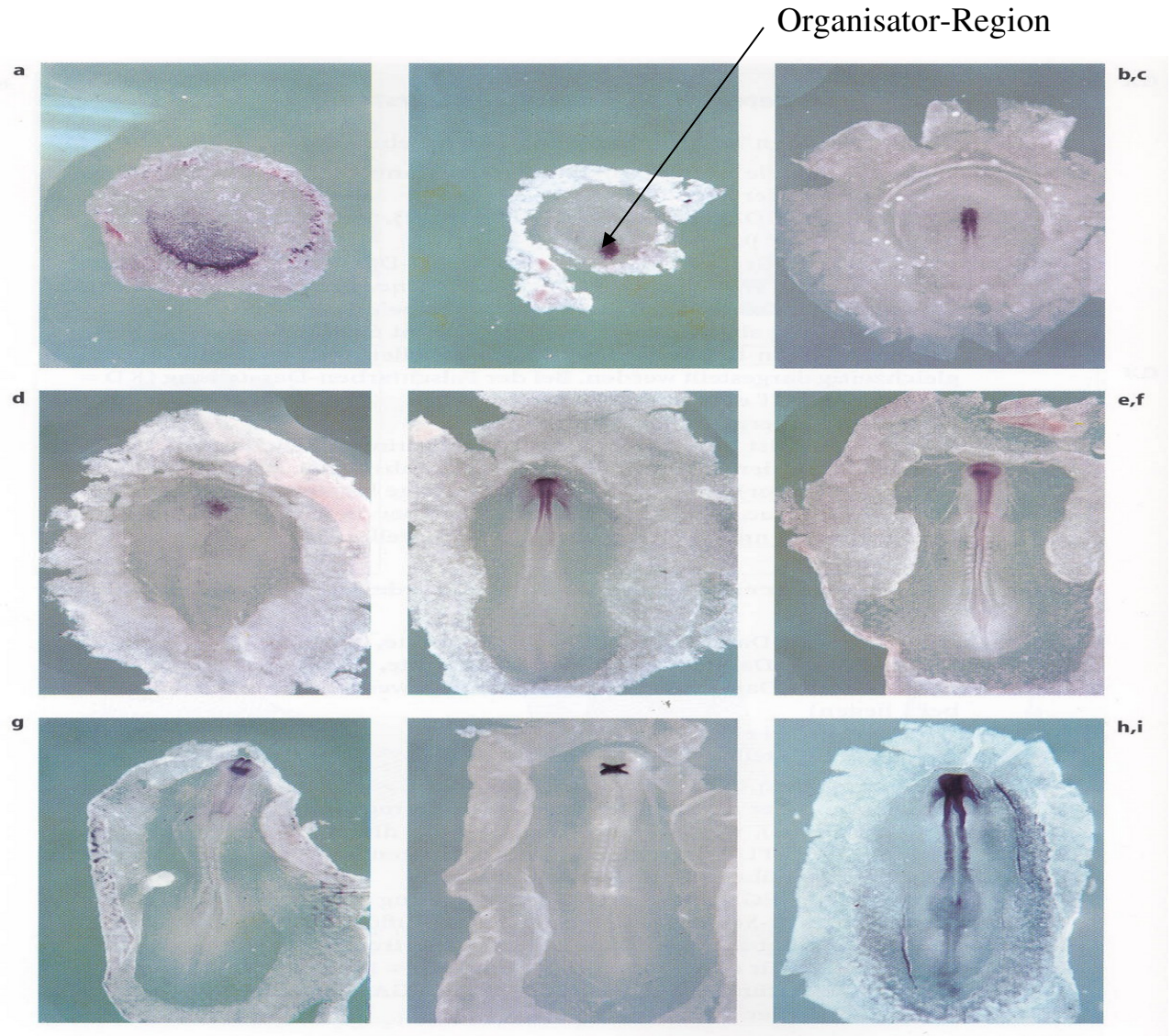
Chicken Embryogenesis

Also in more complex organisms, gradients exist in the development, for example for the activation of genes

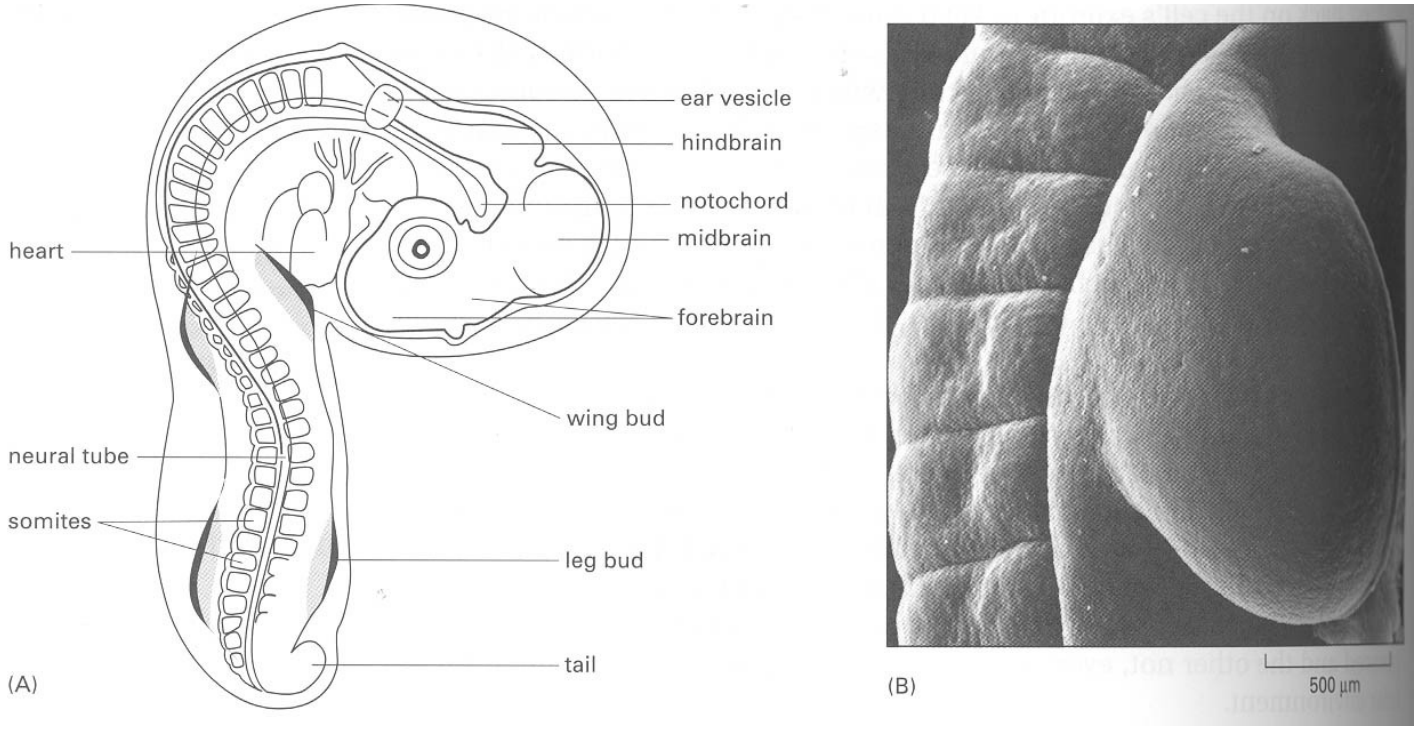


Stages of the development of a Chicken Embryo. (a-h) From a region at the periphery of the blastoderm the Koller-Sichel (ks), cells move into the area pellucida, which leads to a cone-shaped structure. At its cusp, the head is later formed. Starting from the head, the other structures are formed in a wave-like process. We stained the gene oosecoid which is characteristic for the Organisator region. This gene marks initially the Koller-Sichel, later the hard and finally the brain region. (i) a related gene, GSX is initially similarly activated, later however in the posterior regions. The shown phase is similar to the one shown in Figure (e).

taken from: Meinhardt, H., *Wie Schnecken sich in Schale werfen*. 1997, Berlin: Springer Verlag.

Chicken Embryogenesis

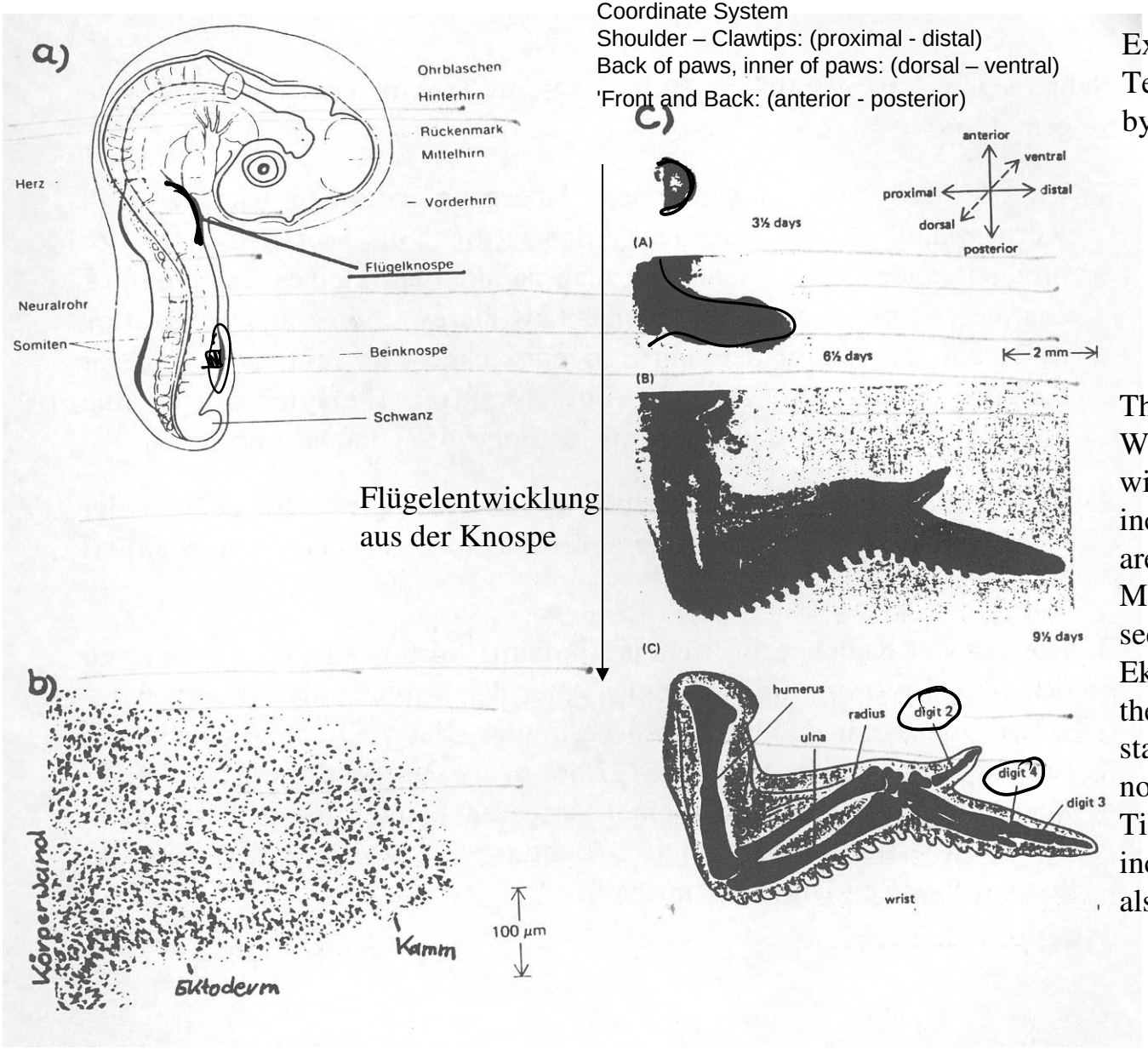
Chicken: Wings and extremities are developed very early on as buds



Chick limb development. (A) A chick embryo after 3 days of incubation, illustrating the positions of the early limb buds. (B) Scanning electron micrograph showing a dorsal view of the wing bud and adjacent somites 1 day later; the bud has grown to become a tongue-shaped projection about 1 mm long, 1 mm broad, and 0.5 mm thick.

taken from: Alberts, V.B., et al., *Molecular biology of the cell*. 3rd ed. 1994, New York: Garland Publishing Inc.

Chicken Embryogenesis



Coordinate System
Shoulder – Clawtips: (proximal - distal)
Back of paws, inner of paws: (dorsal – ventral)
'Front and Back: (anterior - posterior)

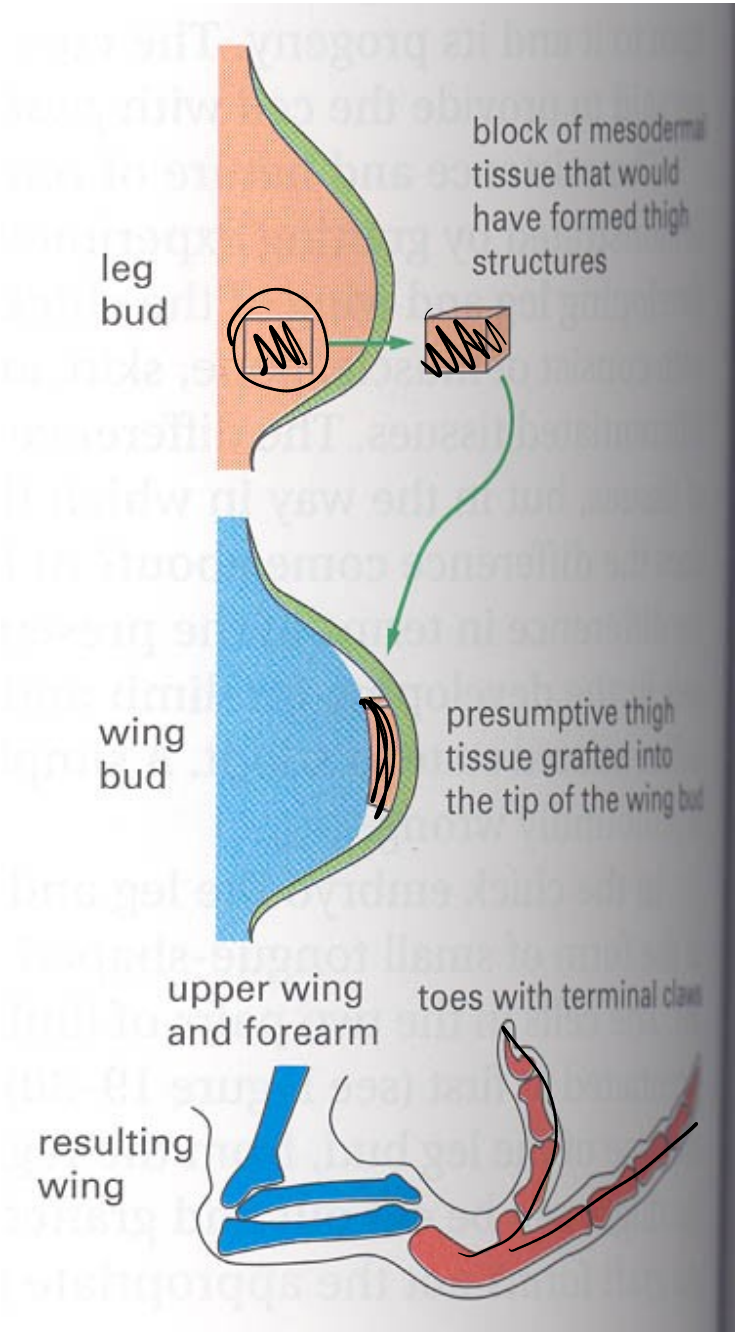
Flügelentwicklung aus der Knospe

Experiment:
Test of the differentiation of cells by **Transplantations**-Experiments

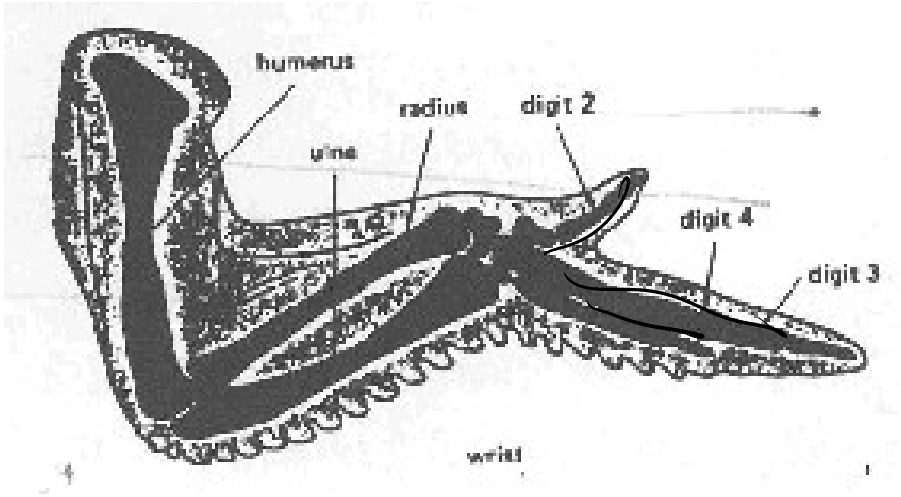
The developlment of Chicken Wings. (a) Positions of leg and wing buds (3 days after incupation). (b) The dark region are stained multipotent Mesenchym cells. It is possible to see the thin outer cell layer, the Ektoderm. The comb is located at the end of the bud. (c) Some stages of development of a normally developing wing bone. Times are given between incubation and observation. We also show the coordinate system.

aus Taken from: Sackmann, E., *Vorlesungsskript Biophysik (3. Auflage)*. 1992, Technische Universität: München.

Chicken Embryogenesis



Example for a Transplantation experiment:
Tissue from a leg bud is taken and inserted into a growing wing bud.
→ Wings develop as leg (with claws)
→ a control substance must exist in differentiated cells which program the cells to develop a leg.

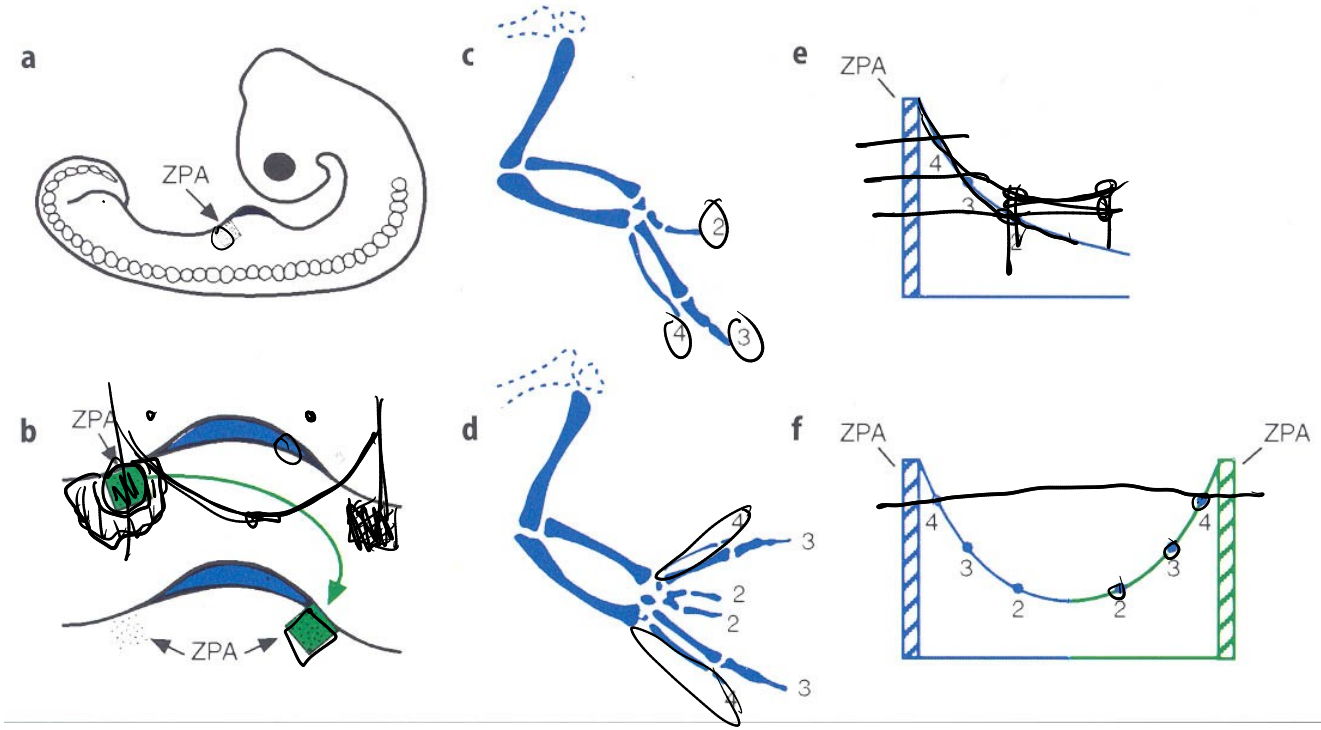


Healty wing: upper arm, lower arm, fingers

Prospective thigh tissue grafted into
the tip of a chick wing bud forms toes

taken from: Alberts, V.B., et al., *Molecular biology of the cell*. 3rd ed. 1994, New York: Garland Publishing Inc.

Chicken Embryogenesis

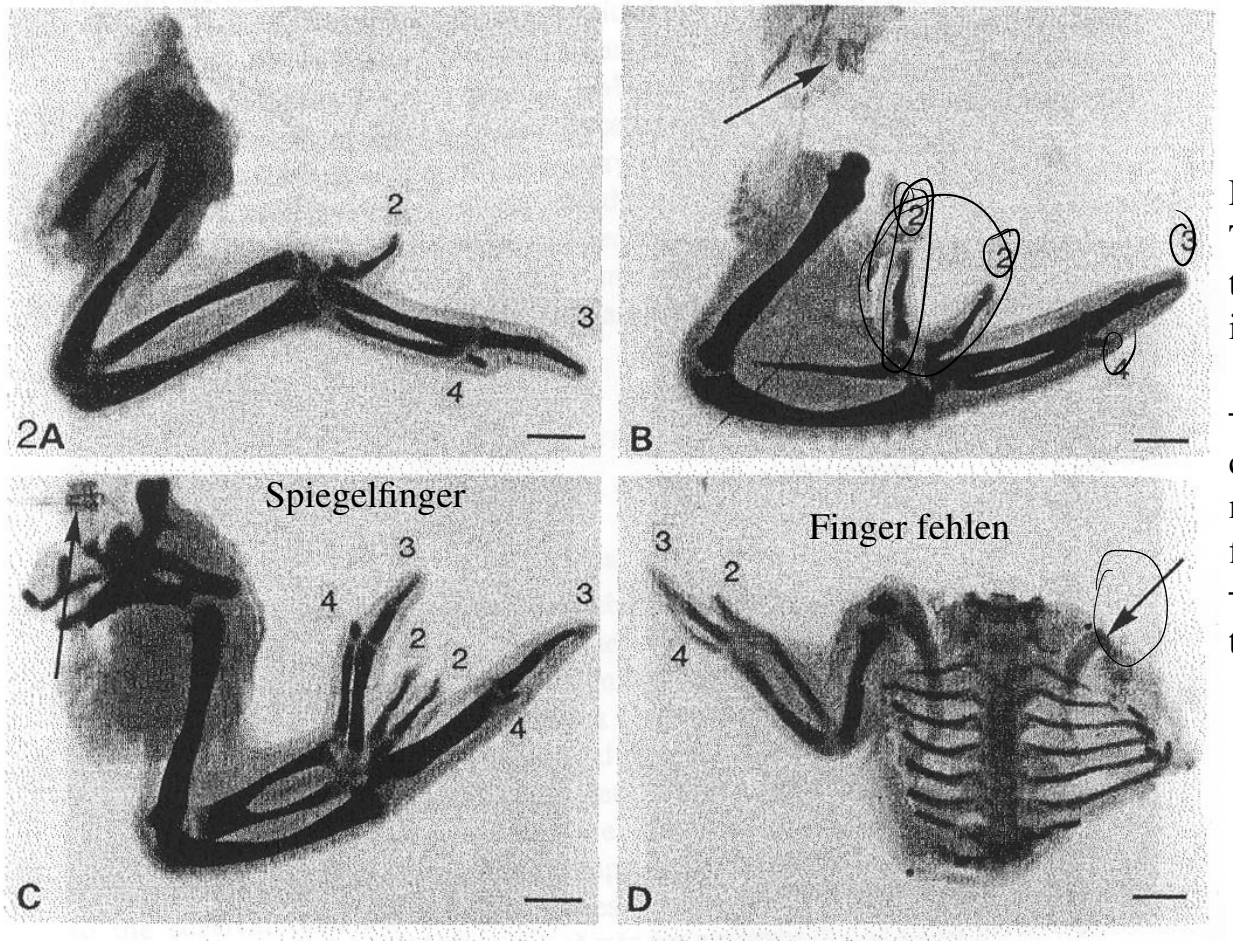


Assumption:
Organisator regions exist from which morphogen gradients emerge. The position in the gradient (i.e. its absolute concentration) determines which cells are differentiated.

Proof of Organisator-Region for the development of members for Vertebrates. (a) Position of zone with polarising activity (ZPA) at the back end of the wing bud of chicken. (b) ZPA-cells are implanted into a second Embryo in front of the wing bud. (c) Normal position of the bones. (d) After transplantation, the bones develop duplicated with a mirror symmetry. Already the implantation of only 100 cells are sufficient. (e,f) Explanation based on the concept of position information based on morphogenic substances. The formation of a graded distribution. Its local concentration determines, which wing bone (2,3,4) is developed. (f) After the transplantation, the symmetrical distribution develops with a mirror-symmetric arrangement of the fingers.

taken from: Alberts, V.B., et al., *Molecular biology of the cell*. 3rd ed. 1994, New York: Garland Publishing Inc.

Chicken Embryogenesis



Example:
Transplantation of **unspecific** tissue impregnated with vitamin A into the wing bud

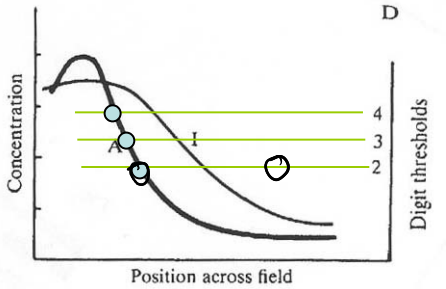
→ Average vitamin A concentration: development of mirror fingers, i.e. too many fingers.
→ High Vitamin A concentration: the fingers are missing.

Examples of results. (A) Normal limb. (B) Limb with one supernumerary digit and slightly reduced radius. (C) Limb with full mirror-image hand and one extra zeugopodal element. (D) Severely reduced limb. Arrow indicates implant, bar = 1 mm.

taken from: Summerbell, D., *The effect of local application of retinoic acid to the anterior margin of the developing chick limb.* Journal of embryology and experimental morphology, 1983. **78**: p. 269-289.

Chicken-embryo - Theoretical Models

normal Wing

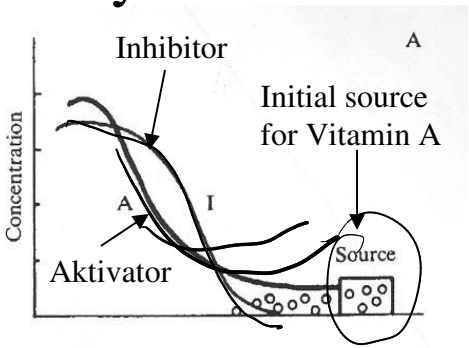


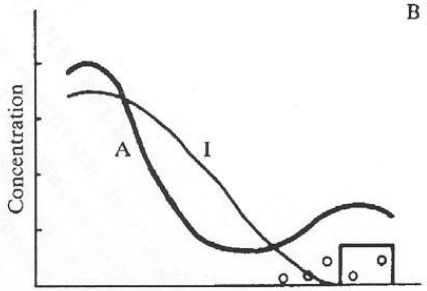
Finger:

4 3 2

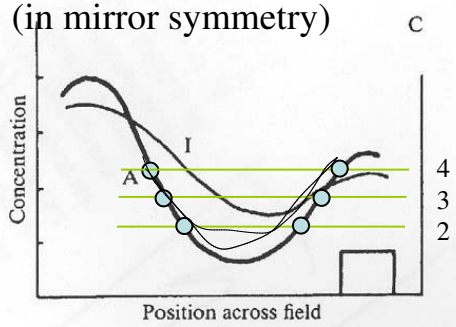
Description of finger sequence:

for each finger a threshold for activator exists: finger 2 is activated at a low level, finger 3 for higher and finger 4 for even higher levels.
The development of finger 3 blocks the creation of finger 2, finger 4 blocks the creation of finger 3.
→ at each threshold a defined finger is developed.





Wing with too many Fingers
(in mirror symmetry)



Finger:

4 3 2 2 3 4

malformed Wing: "Mirrorfingers"

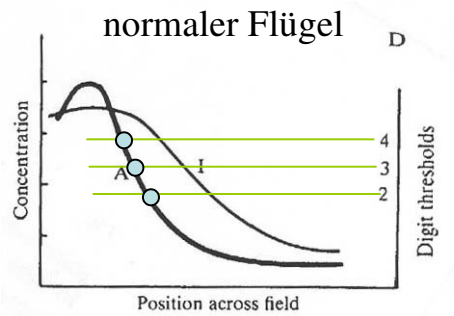
Possible explanation: Vitamin A binds inhibitor and neutralizes locally its effect.
→ Another activator maximum can be built due to reduced inhibition
→ System stabilizes, i.e. the second Activator maxima is persistent after the removal of Vitamin A.

Series of diagrams showing disruption of reaction-diffusion model by local application of retinoic acid (= vitamin A). Heavy line (A) = activator; light line (I) = inhibitor; circles represent retinoic acid molecules. The additional assumption is that Vitamin A binds to the inhibitor. It therefore lowers the level of free inhibitor below the threshold at which the activator can escape from inhibition (A). Activators forms a new anterior peak (B) and reaches a concentration at which it can catalyse production of sufficient inhibitor to eventually neutralize the vitamin A. A new steady state is set up with two stable peaks giving supernumerary elements in mirror-image symmetry (C).

taken from: Summerbell, D., *The effect of local application of retinoic acid to the anterior margin of the developing chick limb.* Journal of embryology and experimental morphology, 1983. **78**: p. 269-289.

Chicken-embryo - Theoretical Models

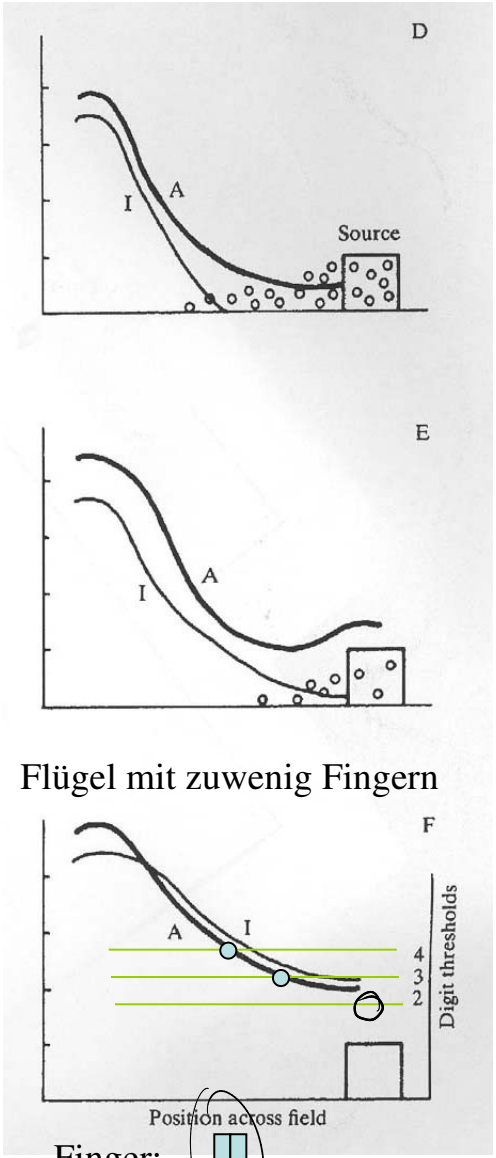
normaler Flügel



Finger:

4 3 2

Series of diagrams showing disruption of reaction-diffusion model by local application of retinoic acid. Heavy line (A) = activator; light line (I) = inhibitor; circles represent retinoic acid molecules. Excess Vitamin A lowers the inhibitor concentration over more of the limb field (D). The whole system escapes from the negative feedback control and activator concentration rises (E). The net effect is to reset the entire reaction diffusion but at a higher base concentration. Digits specified by low concentration ranges are therefore progressively lost giving anterior reductions (F).



Flügel mit zuwenig Fingern

Finger:

4 3

Malformed wing:
missing finger

Vitamin A binds inhibitor and neutralizes locally its effect.

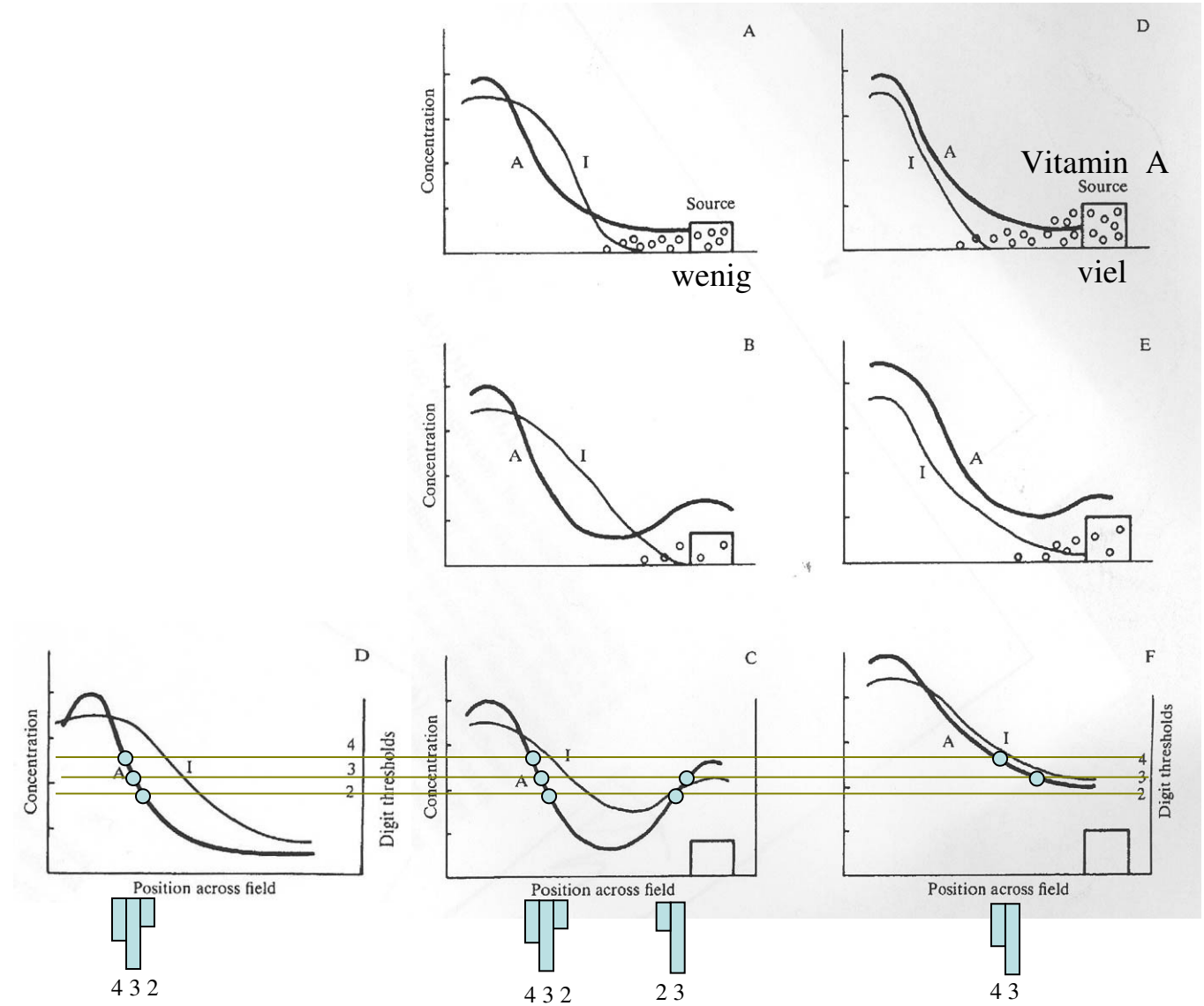
→ A second maximum of activation can develop under the missing inhibition with Vitamin A. The activator level is above the inhibitor level such that the back action is switched off for some time.

→ System stabilizes. however with higher level of activator even for small concentrations of Vitamin A. The low level of activation to make finger 2 is never reached and the finger is missing.

taken from: Summerbell, D., *The effect of local application of retinoic acid to the anterior margin of the developing chick limb.* Journal of embryology and experimental morphology, 1983. **78**: p. 269-289.

Chicken-embryo - Theoretical Models

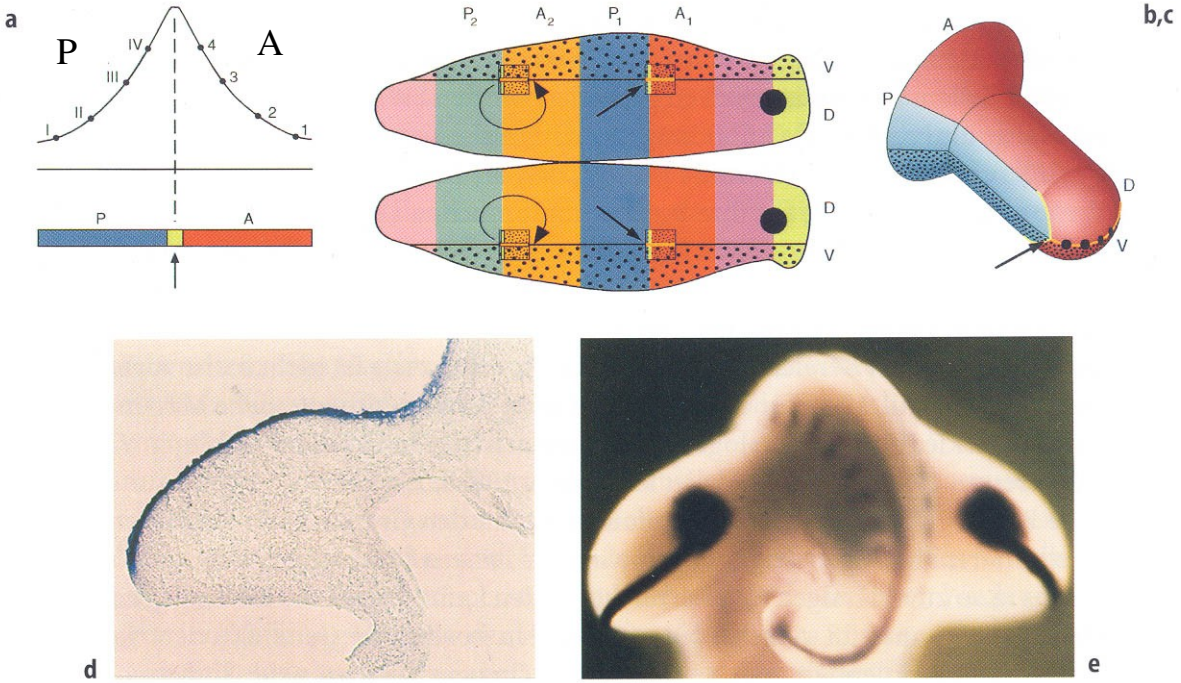
normaler Flügel | too many Fingers | less Fingers



-> Malformed wings can be understood in the basis of the Gierer-Meinhardt Model of activation and inhibition, when the Vitamin A binds the inhibitor and neutralizes its effect.

taken from: Summerbell, D., *The effect of local application of retinoic acid to the anterior margin of the developing chick limb.* Journal of embryology and experimental morphology, 1983. **78**: p. 269-289.

Chicken-embryo - Theoretical Models



Bildung einer Gliedmaßen-Anlage. (a) Modell: Wenn zwei verschieden determinierte Regionen (A und P) zusammenarbeiten müssen,um eine neue Substanz m zu produzieren, so kann deren Produktion nur an der gemeinsamen Grenze stattfinden (Pfeil). Die lokale Konzentration ist ein Maß für die Entfernung von der Grenze. (b) Wenn die Kooperation von zwei Paaren von differenzierten Zelltypen (A/P und D/V) erforderlich ist, so entstehen die Organisator-Regionen an den Schnittpunkten der beiden Grenzen (Rechtecke). In einem zylindrischen Embryo entstehen diese immer in Paaren, eines auf der linken, das andere auf der rechten Seite. Um den unterschiedlichen Drehsinn dieser Schnittpunkte (ovale Pfeile) zu zeigen, wurde die linke Körperhälfte nach oben geklappt dargestellt. (c) Eine Beinknospe während des Auswachsens. Die Finger entstehen entlang der D/V-Grenze (dicke Punkte), der Fingertyp ist von der Entfernung zur A/P-Grenze abhängig. (d) Flügelknospe des Hühnchens. Das Protein Wnt-7a (dunkelblau) ist auf den dorsalen Bereich begrenzt. (e) **Blick vom Schwanz her auf die zwei Flügelknospen. Die Linien entstehen durch Färbung eines Proteins, das an der D/V-Grenze produziert wird (FGF8). Die beiden runden Flecken markieren eine hohe Konzentration von Sonic hedgehog, das an den Schnittpunkten der A/P und der D/V-Grenzen (dicke Pfeile in b,c) entsteht. Sonic hedgehog legt direkt oder indirekt die Reihenfolge der Finger fest (hohe Konzentration: kleiner Finger; niedrige Konzentration: Daumen).** Der vermutlich entstehende Gradient ist nicht sichtbar, da die Konzentration zwischen den Zellen unter der Nachweisgrenze liegt.

How do we generate the left-right symmetry in most animals?

We start with a hollow cylinder in which gradients are developed. The result is a double gradient along the top/down plane.

Additionally, we assume a top-down gradient along the length of the body to create an A-P gradient.

Additionally, we can assume a D-V gradient from front to back.

To develop extremities, enough D+V is necessary

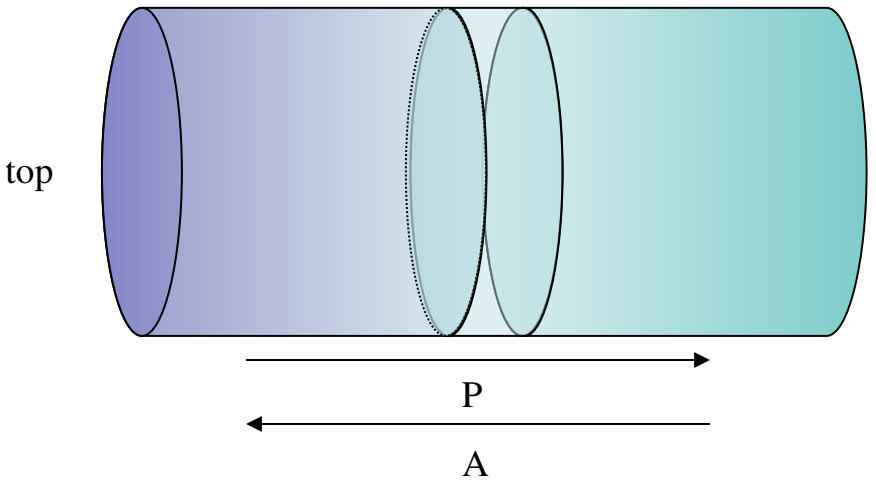
→ Only development at the center between front and back.

→ Only development in the middle in terms of top/down, if addionally sufficient concentrations of

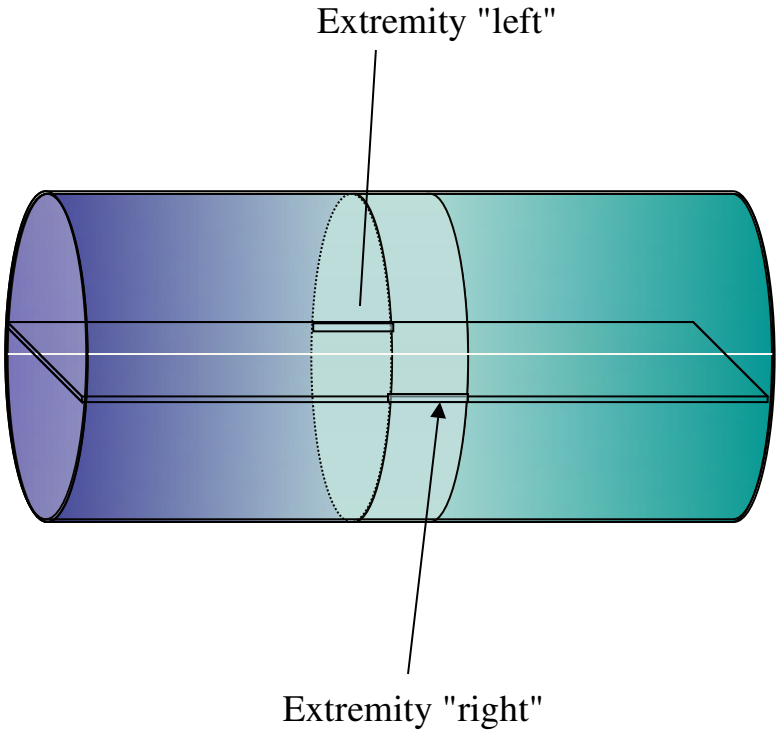
→ Only at two positions extremities can form.

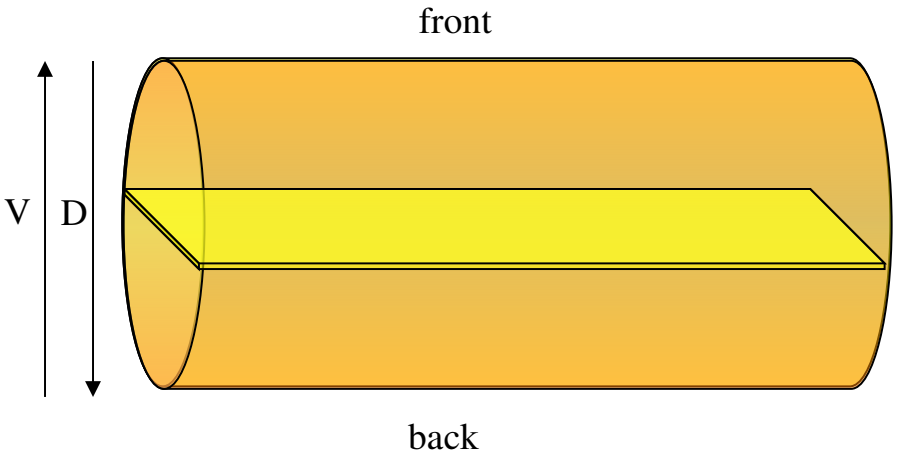
taken from: Meinhardt, H., *Wie Schnecken sich in Schale werfen*. 1997, Berlin: Springer Verlag.

Chicken-embryo - Theoretical Models

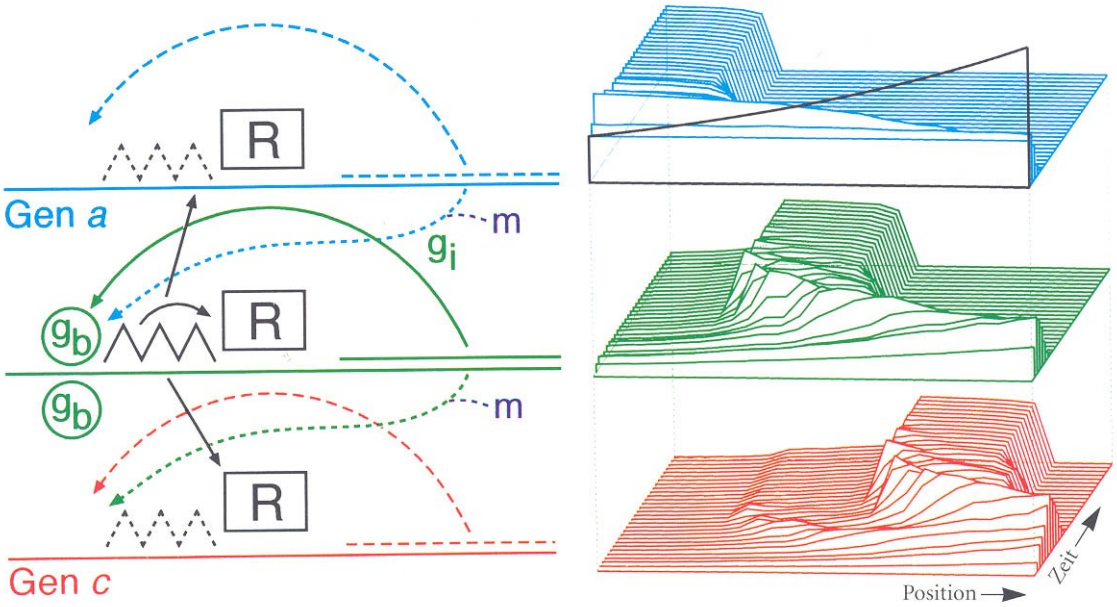


Cross section from two interfaces of double gradients





Morphogenesis as sequence of gene activations

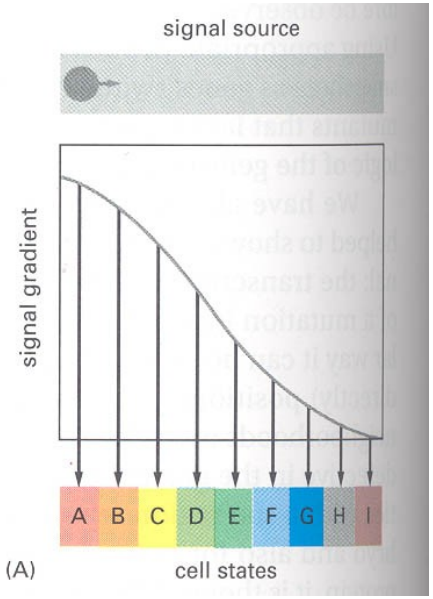


Morphogen-Gradients can trigger the activation of genes.
→ At different positions, genes are activated.
→ expressed proteins have back action on the gene activation to stabilize its effects.

Position dependent gene activation under the influence of morphogenetic gradients. (a) To develop stable cell states, genes must have a non-linear back action on its own stabilization under the competition of other cell states to select only one cell state R. (b) Starting from a homogeneous activation of gene a, the morphogenic gradients will stepwise also activate the genes b and c. Each step will requires a higher concentration of the signal molecule m. The result of the mutual inhibition are sharply defined boundaries between cell states, where either gene a, b or c is activated.

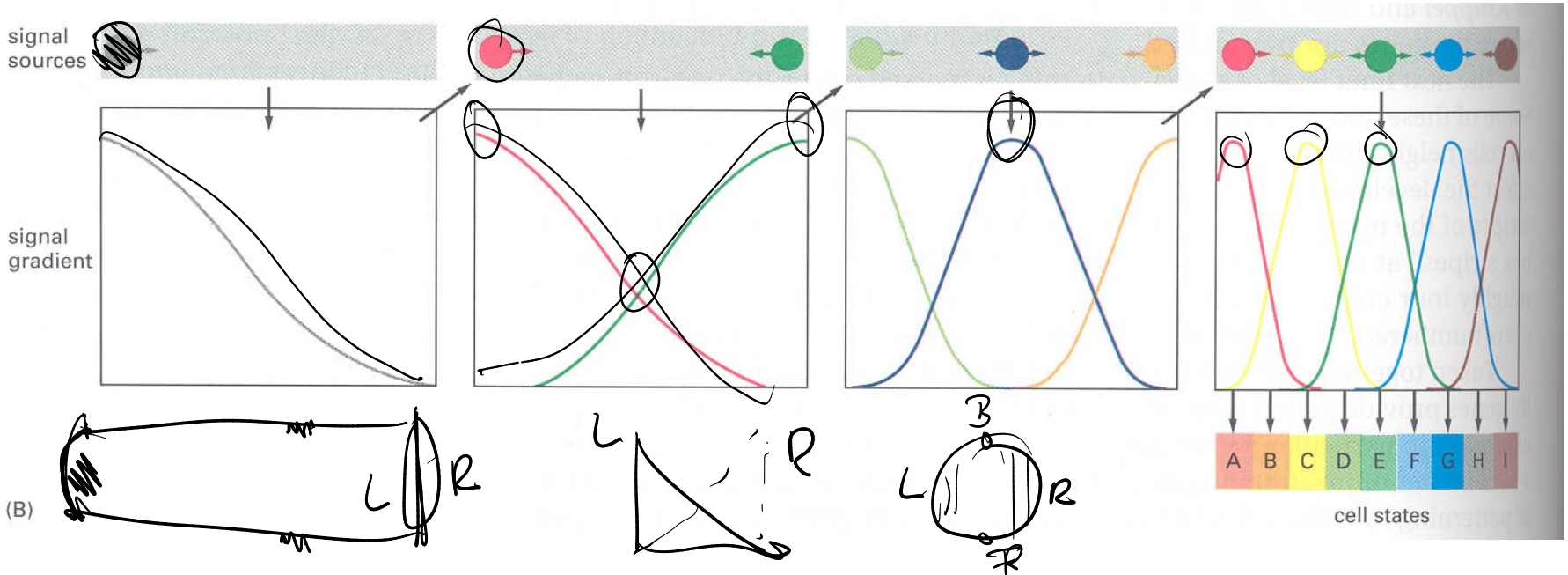
taken from: Alberts, V.B., et al., *Molecular biology of the cell*. 3rd ed. 1994, New York: Garland Publishing Inc.

Some other strategies



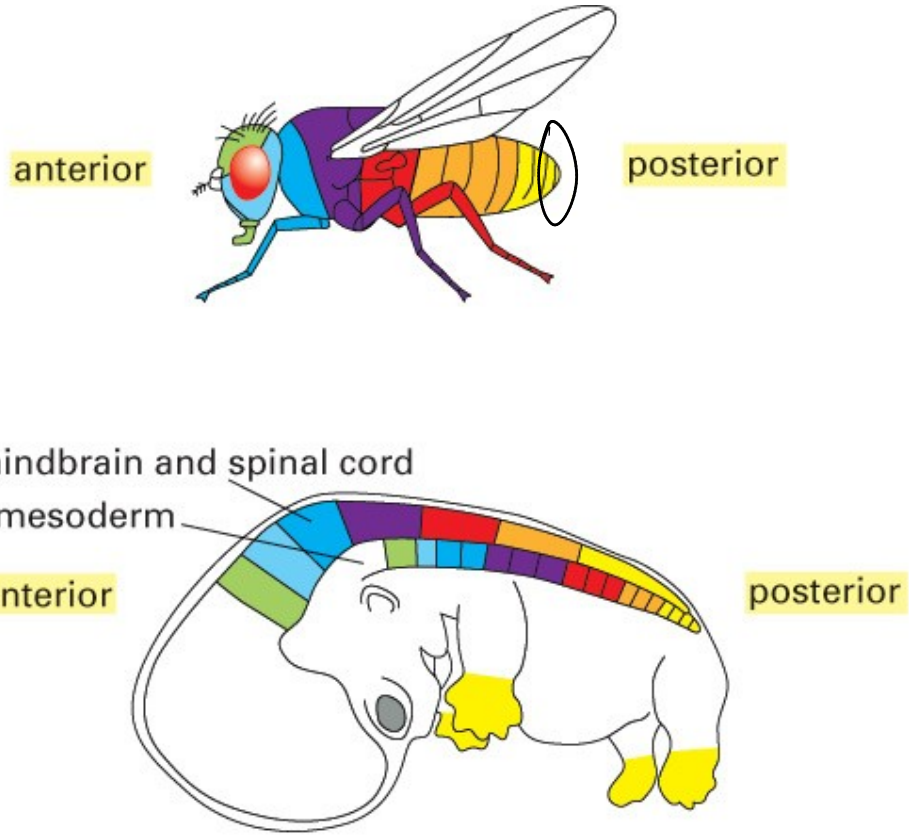
Other effects of Morphogen Gradients

Two strategies for using signal concentration gradients to specify a fine-grained pattern of cells in different states. In (A) there is only one signal gradient, and cells select their states by responding accurately to small changes of signal concentration. In (B) the initial signal gradient controls establishment of a small number of more local signals, which control establishment of other still more narrowly local signals, and so on. Because there are multiple local signals, the cells do not have to respond very precisely to any single signal in order to create the correct spatial array of cell states. Case B corresponds more closely to the strategy of the real embryo.



taken from: Alberts, V.B., et al., *Molecular biology of the cell*. 3rd ed. 1994, New York: Garland Publishing Inc.

Genetics of Pattern formation in Drosophila



21–45 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 21–45 part 2 of 2. Molecular Biology of the Cell, 4th Edition.