factory correspondence with their intact counterparts.

The breakpoints in the autosomal translocation R12 (Fig. 4a) are less certain because there are no morphological differences in the translocated axes as there are in the X-autosome translocations. However, the sharp bend that was taken as the putative breakpoint is evidently correct, as judged both by the close agreement with somatic breakpoint measurements and the correspondence of the reconstructed axes with the nontranslocated axes (Table 1). Moreover, the sum of the translocation axes ($A_{t1} + A_{t2} + B_{t1} + B_{t2} = 13.7$ μm) compares well with the sum of the nontranslocation axes ($A + B = 14.4$ μm). Since small differences could be due to stretching, the data in general indicate that none of the three translocations involves major chromosome losses or gains, supporting the earlier suggestions (*12, 16*) that they are reciprocal. A more precise statement requires further analyses of larger samples.

Autosomal SC formation, an index of synapsis, is apparently not altered significantly in the three translocations. (The separation of autosomal regions near the breakpoint in Fig. 3a may be a technical artifact.) On the other hand, in all cells so far examined, pairing of the X and Y is blocked in R6 (Fig. 2a), but not in R2 (Fig. 3a). Normally, the X and Y are paired distally to form a length of SC (*18*). At early pachytene, the distal third of the X axis and most of the Y are involved; as pachytene progresses, the synapsed region is greatly reduced (Fig. 1b). In R6, the break occurs in the distal third of the X, that is, in the pairing region. In R2, the break is in the proximal portion and evidently does not affect the pairing region. Conceivably, translocations involving a breakpoint within the pairing region of the X could inhibit synapsis with the Y. This is consistent with observations on Ohno and Cattanach's translocation in the mouse (*19*), where occasional Y univalents indicate irregular XY pairing. The breakpoint is genetically close to that of R6 and therefore presumably falls within what has been identified here as the pairing portion of the X. On the other hand, in Searle's translocation (*20*), where the breakpoint is just proximal to the pairing region of the X, the X and Y are observed to pair (*8*). All T(X;A)'s are male sterile, with spermatogenesis ceasing after pachytene (*14, 21*).

Our results verify the nature of the three translocations by a relatively simple procedure that graphically demonstrates the rearrangements at pachytene, permits mapping of the breakpoints, yields additional confirmation of reciprocity, and provides information about meiotic consequences of the rearrangement, such as pairing abnormalities of the X and Y. The method promises to be valuable also in analyzing other chromosomal rearrangements, such as deletions and inversions.

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11. Genetic analysis indicates that in T(X;7)6Rℓ, abbreviated as R6, the autosomal breakpoint is about 2 centimorgans proximal to the *p* locus (*12*), and the X-chromosome breakpoint about 7 centimorgans distal to the *Ta* locus (*13, 14*). Cytologically, by means of banding techniques, the breakpoints have been located in 7B3 and XF1, respectively (*15*). In T(X;7)2Rℓ, or R2, no recombination has to date been observed between the autosomal breakpoint and the *c* locus (*12*); the X-chromosome breakpoint lies between *Ta* and *spf* but closer to the latter (*13, 14*). The cytological breakpoints are at 7D3 and XA2 (or, at 7E1 and XA3), respectively (*14*). The autosomal translocation, T(10;18)12Rℓ, or R12, has its chromosome-10 breakpoint at or very near the Sℓ locus in 10D, with the other breakpoint cytologically located in 18D (*16*).
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Evidence for Abnormal Heart Induction in Cardiac-Mutant Salamanders (*Ambystoma mexicanum*)

Abstract. *Homozygosity for simple recessive gene* c *in axolotl embryos results in the absence of a heartbeat. Gene* c *alters the morphology of the mutant anterior endoderm—the primary heart inductor.*

In an imported stock of dark Mexican salamanders, *Ambystoma mexicanum*, a naturally occurring genetic mutation, designated *c* for "cardiac lethal," has been reported. Homozygous recessive embryos exhibit a total absence of heart contractions even though initial heart development appears normal. Mutant (*c*/*c*) embryos are obtained from matings between heterozygous parents and are first distinguishable from their normal (+/+; +/*c*) siblings at Schreckenberg and Jacobson (*3*) stage 34, when heart contractions first develop in normal embryos. The mutant hearts at this stage, upon gross examination, appear structurally normal but fail to beat. Subsequently, the heart becomes distended and remains thin-walled, and the embryo acquires ascites. Mutants survive for about 20 days beyond the time when the heart would normally have begun to beat; they exhibit normal swimming movements, which indicates that gene *c* does not affect skeletal muscle.

Morphological and biochemical investigations of normal and mutant embryonic hearts from stage 34 (heartbeat stage) through stage 41 (when mutant embryos die) have been reported. Electron microscopy reveals that the normal myocardium has well-organized sarcomeres at stage 34 (the heartbeat stage), and numerous intercalated discs subsequently appear (*4*). By stage 41, the normal myocardium is composed of highly differentiated muscle cells and shows extensive trabeculation (*5*). The mutant myocardium throughout development remains only one-cell-layer thick with no indication of developing trabeculae. Mutant cells at stage 34 have a few sparcely scattered thick (150 Å) and thin (60 Å) filaments along with what appear to be Z bodies. A partial organiza-

tion of myofibrillar components is noted in some mutant myocardial cells at stages 34 to 41; however, distinct sarcomeres are absent (*2*). The results of heavy metomyosin binding, sodium dodecylsulfate–polyacrylamide gel electrophoresis (*6*), and radioimmunossay experiments (*7*) extend these basic morphological observations and confirm that there is a reduction of myosin and tropomyosin in mutant hearts and that actin is present in an unpolymerized nonfilamentous form. Thus, gene *c* in homozygous condition arrests normal heart cell differentiation. The most striking feature of heart morphology resulting from the mutation is a lack of organized myofibrils.

Humphrey (*1*) performed transplants of *c/c* heart primordia into +/+ or +/*c* recipients at Harrison stages 29 and 30 and found the cardiac-lethal defect to be corrected. When reciprocal transplants were performed (+/+ or +/*c* donor heart mesoderm into *c/c* recipients) no heartbeat was observed. Further, parabiosis of normal embryos with mutant siblings at stage 25 did not correct the cardiac deficiency, nor were the normal parabiotic twins adversely affected by this procedure; such conjoined animals lived as long as several months, and the mutant twins, except for lacking functional hearts, appeared normal. These experiments indicate that gene *c* specifically affects the heart and suggest that the failure of normal heart differentiation in cardiac-lethal embryos results from abnormal inductive effects from surrounding tissues. Such effects are restricted to the area of the developing heart and do not circulate in the blood.

As with most vertebrate organs, the heart requires a variety of inductive interactions for normal development; portions of the endoderm and ectoderm have significant interactions with precardiac mesoderm and thus control heart formation. Prospective heart mesoderm has its most important inductive interactions with the anterior dorsolateral endoderm of the early embryo (*8*). This phenomenon has been established experimentally in both amphibians (*8, 9*) and chicks (*10*) and is probably the case for human embryos (*8, 11*). Jacobson and Duncan (*8*) demonstrated these interactions by using hanging-drop cultures for the study of heart induction in the salamander *Taricha torosa*. At stage 16, for example, heart mesoderm alone in culture resulted in the beating of 13 percent of the hearts. With the addition of anterior dorsal endoderm to the mesoderm, 100 percent of the explants beat. On the basis of this and other culture experiments, they were able to demonstrate that the anterior endoderm in salamanders is a specific heart inductor which increases the rate and frequency of heart differentiation. The "active" factor or factors operate in the embryo or when explanted in hanging-drop cultures, and are effectively present in a fraction (from Sephadex column chromatography) of endoderm homogenates. Since, in cardiac-lethal axolotls, the mutant gene seems to exert its effect on heart muscle differentiation by abnormal inductive processes of some kind (*1*), we undertook this study to determine whether there

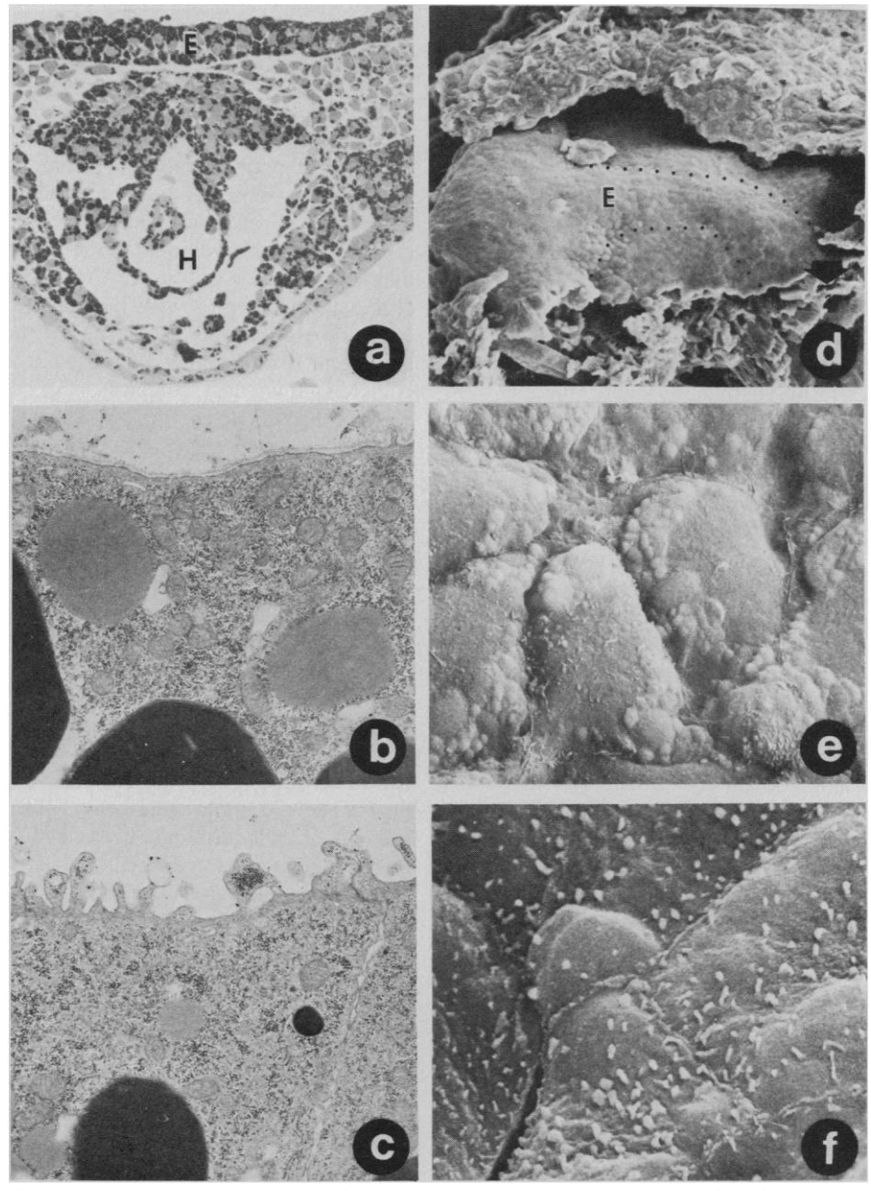

Fig. 1. (a) Light micrograph (× 190) of a transverse section through the heart region of a stage-37 cardiac-lethal mutant salamander embryo. The anterior endoderm (*E*) is located immediately dorsal to the developing heart (*H*). The appearance of the anterior endoderm at this level of resolution is not strikingly different from that of normal siblings. (b) Transmission electron micrograph (× 14,000) of a transverse section showing the luminal portion of an anterior endoderm cell of a stage-35 normal embryo. There are no microvilli. (c) Transmission electron micrograph (× 14,000) of a transverse section through the luminal portion of an endoderm cell of a stage-35 cardiac-lethal mutant embryo. Numerous microvilli have formed, indicating a more advanced level of differentiation than in normal controls. (d) Scanning electron micrograph at low magnification (× 175) showing the luminal surface of the anterior endoderm (*E*) dorsal to the heart tube (arrow) of a normal embryo at stage 35. The approximate location of the heart beneath the endoderm is outlined by dotted lines. There is an intimate association between the endoderm and heart. (e) High-magnification (× 4000) scanning electron micrograph of a portion of the luminal surface of anterior endoderm in a stage-35 normal embryo. Very few microvilli are evident. (f) High-magnification (× 4000) electron micrograph of the luminal surface of anterior endoderm in a stage-35 cardiac-lethal embryo. Numerous microvilli are located on the cell surface, confirming the observations in the transmission electron microscope. It appears that these "gut" cells are more highly differentiated than they are in normal embryos.

were morphological differences between mutant and normal primary inductor tissue—anterior endoderm.

We obtained fertilized eggs from matings between adult axolotls heterozygous for the cardiac-lethal gene. The normal and mutant siblings were reared in the same containers at 18°C until ready for study, at which time they were removed from their jelly coats and prepared for processing. The embryos were staged by using the system of Schreckenberg and Jacobson (*3*). Normal and mutant sibling embryos at stage 34 (about 165 hours after fertilization) and at stage 37 (about 195 hours after fertilization) were studied. Stage-34 embryos were used since this is the first stage that mutant embryos, because they lack heartbeats, can be distinguished from normal ones. Stage-37 embryos are approximately 1 day older. The embryos were anesthetized in 1:10,000 tricaine methanesulfonate (Finquel from Ayerst Laboratories, New York) and were fixed 12 to 24 hours in 3 percent glutaraldehyde or in a glutaraldehyde–formaldehyde–picric acid mixture buffered with 0.10*M* phosphate to *p*H 7.3. The specimens to be used for transmission electron microscopy were fixed again for 2 hours in 1 percent osmium tetroxide buffered to *p*H 7.2 with 0.10*M* phosphate, dehydrated in ethanol, and infiltrated and embedded in Epon. Thick sections were made for orientation, and thin sections of the endoderm were mounted on bare copper grids, stained with uranyl acetate and lead citrate and viewed at 80 kv on an electron microscope (JEOL 100B or Philips 300). Those specimens to be viewed in the scanning electron microscope were dissected after the initial fixation to expose the anterior endoderm in its entirety, with special care being taken to preserve the spatial relationships between endoderm and heart. The pieces of tissue were then fixed for 30 minutes in 1 percent osmium tetroxide buffered to *p*H 7.2 with 0.10*M* phosphate, dehydrated in graded ethanol solutions, cleared in 100 percent acetone, and prepared with a critical-point drying apparatus (Sorvall). The dried specimens were gold coated and viewed in a Cambridge Stereoscan electron microscope at acceleration voltages of 5 or 10 kv.

Histological examination of the endoderm of stage-35 and stage-37 mutant embryos shows no obvious variation from normal. The one-cell-layer-thick anterior endoderm appears to have a close structural association with the heart tube just as in normal embryos (Fig. 1a). Observations made with a transmission electron microscope of thin sections through various levels of normal and mutant anterior endoderm (gut) at stages 35 and 37 were performed. We were careful to take sections from comparable levels through the endoderm of the normal and mutant siblings; four normal and four mutant specimens at each stage were studied. At stage 35, the mutant endoderm cells have many microvilli on their luminal surfaces while the normal endoderm cells have virtually none (Fig. 1, b and c). By stage 37, the normal cells also had some microvilli but still far fewer than mutants; furthermore, the mutant endoderm microvilli appear to be more highly differentiated (*12*) than they are in normal embryos. Scanning electron microscopy of the anterior endoderm from normal and mutant embryos confirms the observations of the transmission electron microscope. After initial fixation, the embryos were dissected to expose the entire luminal surface of the endoderm, which allowed us to make a complete study and comparison of all areas of the endoderm (Fig. 1d). The normal anterior endoderm (Fig. 1e) has many fewer microvilli than does the mutant endoderm (Fig. 1f). The appearance of microvilli on vertebrate intestinal epithelial (gut) cells is an early indication of their specialized differentiation (*12*, *13*). Thus, our morphological data suggest that the anterior endoderm in cardiac-lethal mutant axolotls has differentiated more rapidly than is normal. This finding could indicate that the mutant endoderm has differentiated beyond the point at which it can induce the precardiac mesoderm to finally become contracting heart tissue; the mutant endoderm may even have an inhibitory effect of some kind.

Whatever the final mechanism turns out to be, gene *c* in axolotl embryos alters the anterior endoderm—the primary heart-inducting tissue. At the same time, it prevents final differentiation of the heart, which fails to begin beating.

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Lymphocyte and Fibroblast Chalones: Some Chemical Properties

Abstract. *Experiments with lymphocyte and fibroblast chalones indicate that they are complexed strongly with RNA in tissue extracts; have a molecular weight of less than 10,000 and more than 1,000 daltons, and probably about 5,000 daltons; are strongly cationically charged; and contain mannose and perhaps other carbohydrates.*

Cell-specific and endogenous proliferation inhibitors have been reported for a number of tissues (*1*). These inhibitors were called chalones by Bullough (*2*). Moorhead *et al.* (*3*) claimed that lymph nodes contained a chalone which inhibited lymphocyte transformation and had a molecular weight of more than 40,000 daltons. We have shown that ultrafiltration in the range 50,000 to 30,000 daltons concentrates most of the cell-specific inhibitory activities (chalone) of aqueous extracts of lymphoid tissue toward lymphocytes (*4*). We have also found that by ultrafiltration within this range, most of the cell-specific inhibitor for fibroblast proliferation may be concentrated from the "used" medium in which WI-38 or other diploid human fibroblasts were cultivated (*5*). Aqueous extracts of lung, which contain a large number of fibroblasts, after concentration by ultrafiltration in this molecular weight range, also demonstrate nonspecies-specific, noncytotoxic, and reversible inhibition of fibroblast proliferation in vitro (*6*).