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CHANGES IN SYMMETRY RELATIONS IN *Fundulus* ASSOCIATED WITH THE PRESENCE OR ABSENCE OF CERTAIN PEPTIDES

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Samples were obtained as sodium dodecyl sulfate (SDS) stabilized peptides from eggs and embryos of *Fundulus heteroclitus* (Linn.) under strict conditions of temporal control. Initial separations were made by electrophorsis on ten percent polyacrylamide SDS gel columns under standard conditions (Laemmli, U.K., Nature 227: 680, 1970). It had been previously reported (Schwartz, R.J. and Wilde, C.E., Jr., Nature 245: 376, 1973) that with development there is a shift in synthesis toward proteins of higher molecular weight. An initial pattern obtained in early zygotes formed a relatively stable background sometimes confusing the analysis of the de facto shifts.

We have previously established that terata of specific types can be caused by inhibition of ribonucleic acid synthesis (at specific times at or following fertilization, Wilde, C.E., Jr. and Crawford, R.B., Exptl. Cell Res. 44: 472, 1966). The types of terata expressed are dependent upon the time of inhibition pulse initiation and not on pulse duration. Similar data have been published and similar terata demonstrated by the use of an inhibitor of protein synthesis (pactamycin). The time period data are almost identical (Crawford, R.B. and Wilde, C.E., Jr., Exptl. Cell Res. 77: 489, 1966; Crawford, R.B., Wilde, C.E., Jr., Heinemann, M.H. and Hendler, F.J., J. Embryol. exp. Morph. 29: 363, 1973).

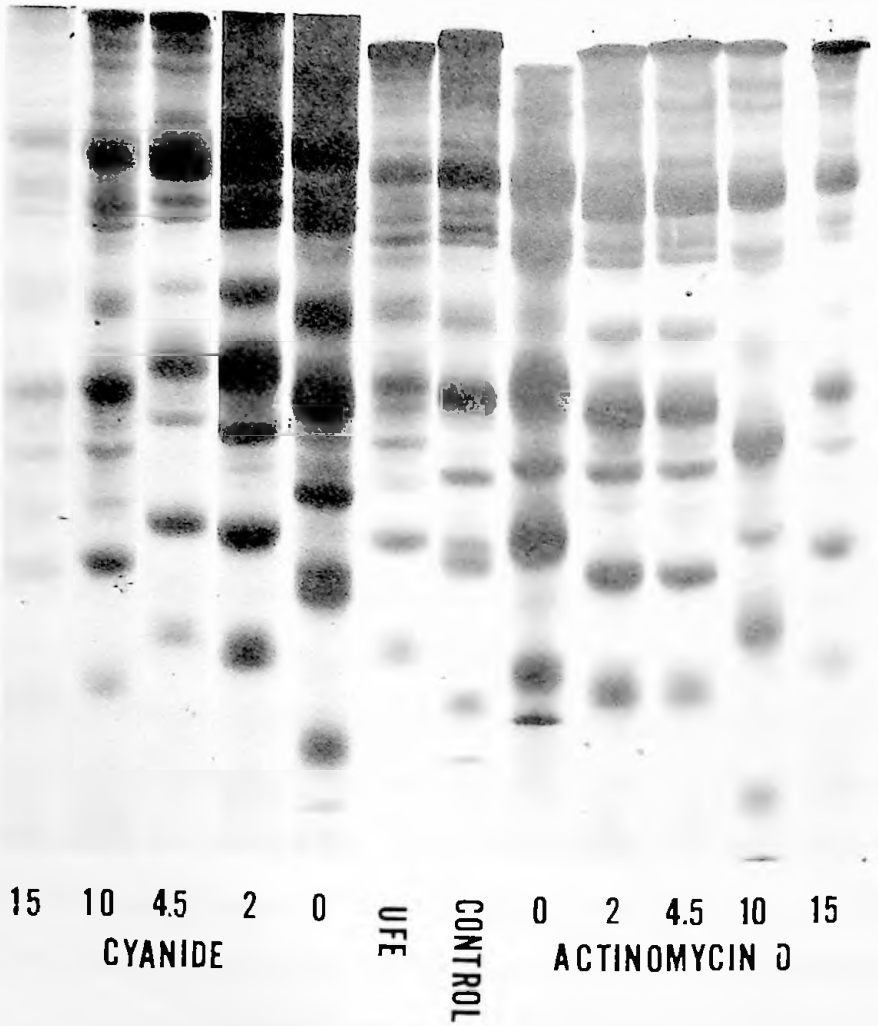
All anomalies induced by these treatments are permanent although the macromolecular synthesis inhibition causal to the anomaly can be restored to

normal upon removal of the inhibitor.

CN^- is an absolute inhibitor of all RNA synthesis. The zygotes develop up to the high blastula normally but then cease their morphogenesis. Upon removal of the inhibitor both normal morphogenesis and normal RNA synthesis are resumed. Actinomycin D is a well known inhibitor of DNA dependent RNA synthesis. We have previously reported its effects in this system. (Wilde, C.E., Jr. and Crawford, R.B., Exptl. Cell Res. 44: 471, 1966; Crawford, R.B. and Wilde, C.E., Jr., Exptl. Cell Res. 44: 489, 1966). Our experiments to be outlined below take advantage of these previously published facts.

SDS peptides were prepared from isochronously fertilized eggs which had been pulsed with ^3H valine (1μCi/ml, spec. act. 1.3Ci/mM, New England Nuclear) for 24 hours or until stage 10, Oppenheimer (Oppenheimer, J., Anat. Rec. 68: 1, 1937) was reached. The time of onset of inhibitor pulse was: Fert. + 0 min.: Fert. + 2 min.; Fert. + 4.5 min.; Fert. + 10 min.; Fert. + 15 min. The radioisotope pulse was concurrent. Triplicate series were run for each period, namely control, actinomycin D (20μgm/ml) and CN^- (2×10^{-3} M) all in 50% millipore filtered sea H_2O. Nine complete experimental units were carried out with eggs of 95% + fertilization rate. At stage 10 (high blastula) the zygotes were washed and SDS peptides were prepared by methods published in Schwartz, R.J. and Wilde, C.E., Jr. (Nature 245: 376, 1973). Preliminary examination of gel runs (via densitometry) with Coomassie Blue staining indicates a thoroughly repeatable and consistent series of changes in banding pattern depending upon the type of inhibitor insult and the time of its onset.

Controls in all these cases were untouched by the drug. Thus their gels represent a portion of the peptides of normal development unimpeded by insult (isotopic amino acids added at proper pulse onset times used in the experimental series will upon future analysis give evidence in the controls of the



time of onset of the synthesis of particular peptides, Schwartz, R.J. and Wilde, C.E., Jr., Nature 245: 376, 1973. This material will be reported in detail elsewhere).

Experimental results are summarized below and in Figure 1. Reference gels were UFE and Control. Since UFE represents the peptides present in ripe, unfertilized eggs and since Control represents peptides of blastula-gastrula (stage 10) or the configuration resultant from 24 hours of normal synthesis, it would be expected that major differences would be apparent as they are. In addition UFE will be most like peptides from CN^- treated embryos especially

where the "window" permitting a period of normal metabolism is 0 min. (Fert. + 0), as during the inhibitor pulse period there would be no RNA synthesis (Crawford, R.B. and Wilde, C.E., Jr., Exptl. Cell Res. 44: 489, 1966, i.e. through the 24 hour period of development to stage 10). This result is evident in Figure 1. The pattern of peptide banding in CN^- treated zygotes in specimens with a two minute window is somewhat different from that in the UFE series. This is true for specimens where a 4.5 minute window was used. However these two are similar to each other. The experiments with 10 and 15-minute windows remain similar in part to UFE and different from control, reflecting a minimum 23-3/4 hours of synthesis in the control which had not been experienced in the experimental sequences. The subtle differences noted form a graded series reflecting the gradual increment of syntheses during the few minutes after fertilization when this was permitted by experiment design.

When similar pulses of actinomycin D are delivered there is no morphogenetically meaningful protein synthesis for the period of the pulse and during a short recovery period although the inhibitor reduced RNA synthesis by approximately 50 percent. Still the peptides from actinomycin D series with a 0 window should be most similar to UFE and least similar to control. This was noted. Furthermore peptides from actinomycin D treated embryos were different among themselves, 0 window being different from two minutes, 4.5 minutes, 10 minutes, and 15 minutes. Two and 4.5 minute were closely comparable as were 10 and 15 minute. The 2 and 4.5 minute were different from the 10 and 15 minute. The 10 and 15 minute window series tended to be most like control of all cases examined.

Embryos which develop from experiments with actinomycin D where the window is zero develop as balls of cells without axes of symmetry organo genesis. Their peptide patterns remain like those of UFE when extracted at

stage 10.

Embryos which develop from experiments with actinomycin D with a 2 or 4.5 minute window are barely axiate and anencephalic. These extracts have a quite specific peptide pattern somewhat different from UFE and actinomycin D with 0 window. Embryos which develop from experiments with 10 and 15 minute windows are microcephalic at worst and often apparently normal. Their extracted peptide patterns are most like those of control and least like UFE.

The preliminary data reported here correlate the changes in peptides synthesized at or not synthesized at specific times in the early minutes following fertilization with specific failures in morphogenesis. These failures result in permanent anomalies. Since after relief of CN^- treatment morphogenesis is normal and peptide synthesis is restored, the retention of UFE-like peptide patterns must reflect only a general and temporary "marking time." The terata from the temporal failure of synthesis due to actinomycin D inhibition of the synthesis of certain RNAs is strong evidence of the requirement at specific times for the synthesis, upon appropriately encoded polyribosomes, of morphogenetically meaningful specific peptides. These function in normogenesis. In their contemporary absence abnormal development is assured perhaps through failure of synchronization.

Experiments to allow comparison of isochronous series with CN^-, where normal development can be resumed, and actinomycin D, where it cannot, are currently in progress and should act as a preliminary screening mechanism for the further characterization of these macromolecules. Further experiments are planned to isolate them.

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