experimental results illustrate the possibility of developmental control by systems using a transmitter and rectifying signal transmission, and we suggest that such control may be widespread. Electrophoretic investigation of other developing systems, and particularly those where other periodic phenomena are known, should be illuminating. These range from periodic movements in neurulation and gastrulation (*15*) to periodic cell division in blastulas and periodic nuclear division in *Physarum polycephalum* (*22*), and in some syncytial tumors (*23*).

It should be possible to control all these periodic cellular processes by using similar, but appropriately modified, external signals delivered from a source of cellular dimension such as a microelectrode tip. Moreover, it should be possible to elucidate the several functional elements of a developmental control system and to establish the order in which functional competence emerges for each element during development. For example, our present experiments with *D. discoideum* show unequivocally that the several components of the aggregation control system develop sequentially during interphase. These results are summarized in Table 1 together with related results by earlier workers.

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16. Centrifugation was in distilled water at 4°C for two periods of 2 minutes at 650*g*.
17. The solution contained 0.025*M* KH_2PO_4, 0.0125*M* K_2HPO_4, 0.0001*M* $MgCl_2$, 0.0001*M* $CaCl_2$.
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Mammary Carcinoma: A Specific Biochemical Defect in Autonomous Tumors

Abstract. *Rat mammary carcinoma (R3230AC) which does not regress after ovariectomy has a markedly reduced amount of cytoplasmic estradiol binding protein. Cytoplasm from the tumor fails to interact with estradiol sufficiently to permit estradiol binding to tumor chromatin. This defect can be corrected in vitro by substituting cytoplasm, containing the binding protein, from rat uterus, thus permitting estradiol binding to tumor chromatin. The results indicate that the hormonal autonomy of this carcinoma is due to a lack of estradiol binding protein and not to the inability of estradiol to interact with the cell genome.*

We recently reported that dimethylbenzanthracene-induced mammary carcinomas which regress after ovariectomy contain a specific cytoplasmic 17β-estradiol binding protein (EBP), whereas mammary carcinomas which continue to grow after ovariectomy lack this protein (*1*). In further consideration of this defect in autonomous mammary tumors we found a 90 percent reduction in cytoplasmic EBP in a transplantable rat mammary carcinoma (R3230AC) which does not regress after ovariectomy but has retained the capacity to respond partially to estrogen by stimulating certain enzyme activities (*2*). In classical estrogen target tissues or hormone-dependent mammary carcinoma, estrogen enters the cell, interacts with the specific EBP, migrates to the nucleus, and then binds to chromatin. Since the R3230AC tumor fails to accumulate intranuclear estradiol significantly, it became of interest to study the in vitro binding of estradiol to chromatin prepared from R3230AC tumor nuclei in the presence and absence of EBP. These experiments were undertaken to determine whether the estradiol autonomy of these cells is due to a lack of EBP or the inability of the genome to interact properly with the hormone-binding protein complex. We now report that, in the presence of R3230AC cytoplasm, estradiol fails to bind R3230AC chromatin. In the presence of uterine cytoplasm which contains abundant EBP, estradiol binds appreciably to R3230AC chromatin.

Chromatin from R3230AC tumor nuclei was prepared by techniques described (*3, 4*). The ratio of histone protein to DNA and that of nonhistone protein to DNA were 0.90 to 1.12 and 1.11 to 1.42, respectively, as determined

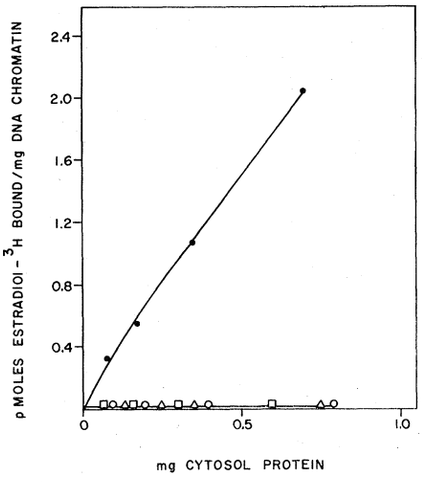

Fig. 1. Binding to chromatin of [^{3}H]estradiol in the presence of increasing concentrations of cytoplasm from rat uterus (solid circles), R3230AC tumor (open circles), rat brain (squares), and rat muscle (triangles). The incubation mixture included R3230AC chromatin (DNA content 44 μg), cytoplasm which had been previously incubated as described in the text. The concentration of chromatin in this experiment was too high to demonstrate saturation by uterine EBP.

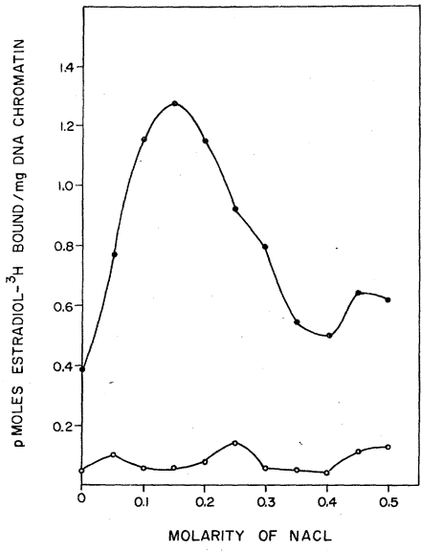

Fig. 2. Effect of salt concentration of R3230AC chromatin binding of [^{3}H]estradiol in the presence of cytoplasm from rat uterus (solid circles, 0.45 mg of cytosol protein per tube) and rat muscle (open circles, 0.44 mg of cytosol protein per tube). Conditions are described in the text, except for the NaCl concentration of the incubation.

by the method of Spelsberg and Hnilica (*3*). Cytosol fractions from rat muscle, brain, uterus, and R3230AC tumor were prepared from the 105,000*g* supernatant of tissue homogenates (*1*) and incubated with 17β-[6,7-^{3}H]estradiol (40 curie/mmole) (^{3}H E_2) for 60 minutes at 4°C. Portions of cytosol plus $^3HE_2$ were then incubated for 30 minutes at 22°C with R3230AC chromatin (DNA content, 25 to 50 μg). The incubation mixture contained 0.15*M* NaCl and

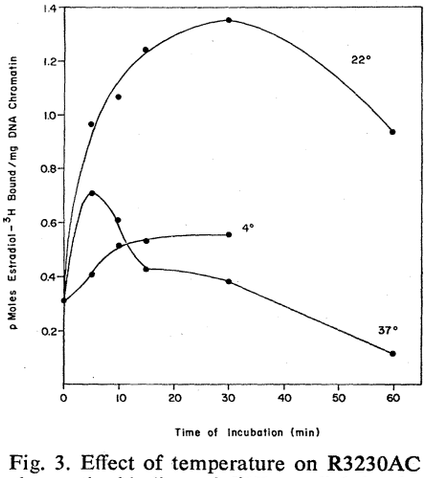

Fig. 3. Effect of temperature on R3230AC chromatin binding of [^{3}H]estradiol in the presence of rat uterus cytoplasm. Conditions are described in the text, except that the tubes were incubated at the indicated time and temperature.

0.01*M* tris-HCl (*p*H 7.4) in a final volume of 0.5 ml. The reaction was stopped by centrifuging the chromatin at 2000*g* at 4°C and washing the pellet three times with 0.15*M* NaCl in 0.01*M* tris-HCl (*p*H 7.4). The pellet was then transferred to a Millipore filter (0.45 μm pore size, 24 mm) and washed under suction with 0.15*M* NaCl, 0.01*M* $MgCl_2$ and 0.01*M* tris-HCl at *p*H 7.4. The filters were then placed in toluene PPO–POPOP scintillation fluid in a Beckman scintillation counter. After the radioactivity had been measured the filters were dried, and the DNA from the filters was quantitated. These procedures have been described (*3*, *4*).

The complex of $^3HE_2$ and cytoplasm from R3230AC tumor, muscle, and brain failed to bind to R3230AC chromatin (Fig. 1). This failure is consistent with the fact that these tissues contain little or no EBP and that estradiol administered in vivo to rats bearing R3230AC tumors does not significantly accumulate in the nucleus. In order to see whether the lack of $^3HE_2$ binding to R3230AC chromatin is entirely attributable to the relative lack of EBP in this tumor, or whether the chromatin is inherently unable to bind $^3HE_2$, we studied rat uterine cytosol, which is known to contain abundant EBP (about 0.06 pmole $^3HE_2$, specifically bound, per milligram of cytosol protein). This is approximately 30 times the amount of EBP found in the R3230AC tumor cytosol (*2*). In Fig. 1, we see that the complex of $^3HE_2$ and rat uterine cytosol demonstrates remarkable binding to R3230AC chromatin.

The binding process is very dependent upon salt concentration (Fig. 2). In the range of physiological salt concentration, optimum binding occurs. If NaCl is omitted or is present in great excess, much less binding occurs.

The effect of temperature on $^3HE_2$ and chromatin interaction is illustrated in Fig. 3. In several experiments incubation at 4°C resulted in very little time-dependent binding. At 37°C we always found an initial rise in binding followed quickly by a time-dependent fall to baseline levels. We interpret the 37°C data as evidence of the liability of the EBP at elevated temperatures as described by others (*5*). At 22°C, a temperature at which the EBP is stable for several hours, we find optimum binding. This temperature-sensitive interaction is consistent with other observations that intranuclear accumulation of $^3HE_2$ does not occur at 4°C (*6*). The above data are clearly consistent with the studies of progesterone binding to chick oviduct chromatin which demonstrate an absolute requirement for cytoplasmic progesterone binding in order for progesterone to specifically bind oviduct chromatin (*4*, *7*).

We have shown that the R3230AC mammary carcinoma during neoplastic transformation has lost the EBP normally found in both ovarian dependent mammary carcinoma and in estrogen target tissue. We proposed that this lack of EBP does not permit the full normal consequence of estrogen action in this tissue (see *2*) and may be related to the lack of tumor regression following ovariectomy. Our data more clearly localize this biochemical defect by demonstrating that providing EBP (cytosol) from an estrogen-dependent tissue permits the binding of $^3HE_2$ to R3230AC tumor chromatin. Our results suggest that chromatin of these tumor cells possesses the capacity for extensive interaction with the $^3HE_2$–EBP complex observed for estrogen target tissues both in vivo and in vitro. Consequently the autonomy of these transformed cells is probably due to the lack of the cytoplasmic estradiol binding protein common to normal target tissues and hormone-dependent mammary tumor cells. Although we have shown the absolute requirement for specific EBP in this binding reaction, we have not yet identified the component of chromatin that participates in this interaction.

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