

solution which was titrated with a barium chloride solution using a thorine indicator. The flow rates were measured with flow meters to 1 l/h for air and 0.01 l/h for the sulphur dioxide-argon mixture. The stream of air was allowed to pass through a system of water-containing flasks in order to saturate it with water vapour. Iron samples of high purity were carefully polished chemically and were then exposed to an atmosphere containing 0.1–1.0 ppm sulphur dioxide. The excess of sulphur dioxide was absorbed in a flask containing hydrogen peroxide solution. After an exposure of 5–20 h, the oxidation chamber was filled with 0.1 M hydrochloric acid and the soluble corrosion products were dissolved while a stream of argon was passed through the solution. The solution was then transferred to the round flask where remaining sulphur dioxide, and possibly even hydrogen sulphide, was removed by boiling *in vacuo*. Samples were taken from the solution and the sulphate was precipitated with barium chloride. The precipitate was analyzed with a Geiger-counter using a sample with known concentration as reference.

The results indicate a high percentage of sulphate in the corrosion products (see Table 1) in agreement with previous experiments.¹ The measured percentages of sulphate can be regarded as constituting the lower limit, since the corrosion products were dissolved under evolution of hydrogen gas, a consequence of the reaction between iron and hydrogen ions. The reproducibility is rather low, probably due to insufficient control of humidity and sulphur dioxide concentration.

Experiments are now being performed under a more rigorous humidity control, and construction materials other than iron are also being investigated.

1. Vannerberg, N. G. and Sydberger, T. *Corrosion Sci.* **10** (1970) 43.

Received March 3, 1970.

Interactions between RNA and DNA at Nucleohistone Formation

IVAR AGRELL

Zoophysiological Institute, University of Lund, Lund, Sweden

An earlier investigation dealing with the separate behaviour of DNA and RNA at nucleohistone formation in model experiments¹ has now been extended to demonstrate the mutual behaviour of the two nucleic acids when competing for histone.

The principal method used has been described elsewhere.¹ High molecular thymus DNA (Sigma, Type I) and yeast RNA (Sigma, Type XI) were utilized. Thymus histone fractions F_1 , F_{2a} , F_{2b} , and F_3 were prepared in our laboratory. Stock solutions were made separately of histone and of RNA+DNA mixtures in 0.12 M NaCl buffered to pH 5 with 0.01 M acetic acid-acetate in concentrations of 1 mg/ml. The nucleic acids were mixed in series of reciprocal concentrations from 0.1 mg RNA+0.9 mg DNA to 0.9 mg RNA+0.1 mg DNA (1:9, 1:4, 1:2, 1:1, 3:2, 2:1, 4:1, 9:1; RNA+DNA=1 mg/ml). Each of these mixtures were in their turn mixed with the F_{2b} stock solution, in duplicate series of reciprocal concentrations from 0.9 mg nucleic acids+0.1 mg histone to 0.1 mg nucleic acids+0.9 mg histone (9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9; histone+nucleic acids=1 mg/ml). After 20 min the precipitates formed were centrifuged at 4000 *g* for 30 min, washed with NaCl buffer and then dissolved in 1 M NaCl. The ultraviolet absorption curves for each precipitate and supernatant were obtained spectrophotometrically using a Unicam automatic scanner. The amounts of histone and nucleic acids were measured through differential readings at two wavelengths. DNA was estimated with the diphenylamine reaction and the amount of RNA was calculated as the difference. One such experimental series is exemplified in Fig. 1. Complementary but less complete series of tests were made for other histones and for ribosomal RNA,² s-RNA (Sigma, Type III) and single stranded DNA (Sigma, Type I, heated to +100°C for 10 min and rapidly cooled down). For a reliable comparison of the behaviour of the isolated nucleic acids with their conduct when mutually interacting, the RNA and DNA were further tested

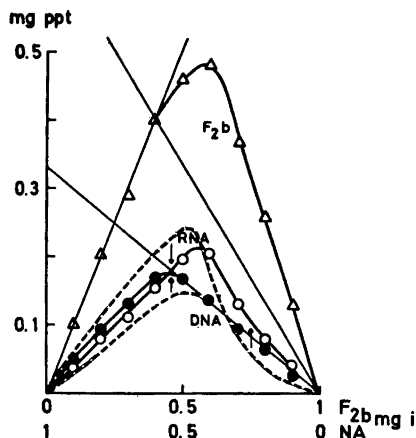


Fig. 1. The DNA, RNA, and F_{2b} precipitated along a reciprocal histone nucleic acid concentration gradient exemplified by a test series using an initial RNA:DNA ratio = 2:1, ● = DNA, ○ = RNA, △ = F_{2b} . Vertical axis: mg precipitate. Horizontal axis: initial (i) amounts in reaction mixture, mg/ml. Straight lines: DNA, RNA, and F_{2b} added. The dotted curves represent the expected precipitation of DNA and RNA according to the test series made with DNA and RNA separately by combining stock solutions of the nucleic acid in varied concentrations with the histone stock solution of constant concentration. An expected amount of precipitate should be formed, when the two nucleic acids are precipitated by histone independently of each other and consequently engage an amount of histone proportional to their respective amounts added and thus show no competition or other interaction. For F_{2b} the found and expected curves coincide.

separately against the F_{2b} gradient using stock solutions of 0.1–1.0 mg/ml of each nucleic acid. In these tests, at the different histone: nucleic acid ratios used, the reactants always precipitate in proportion to the amount added. These values were regarded as representing the expected precipitation including the anticipation that, when combined, the nucleic acids share the histone in proportion to their amounts and thus without special interaction.

It was found that in the nucleohistone precipitates, the amount of histone was practically independent of the initial RNA:DNA ratio. This is expected, because

separately DNA and RNA have almost the same capacity to precipitate F_{2b} . Thus any change found in nucleoprotein composition can concern only an altered contribution of the two nucleic acids. The results from all the test series are combined in Fig. 2. It is evident that relative precipitation maxima and minima appear, *i.e.* values contrary to those expected.

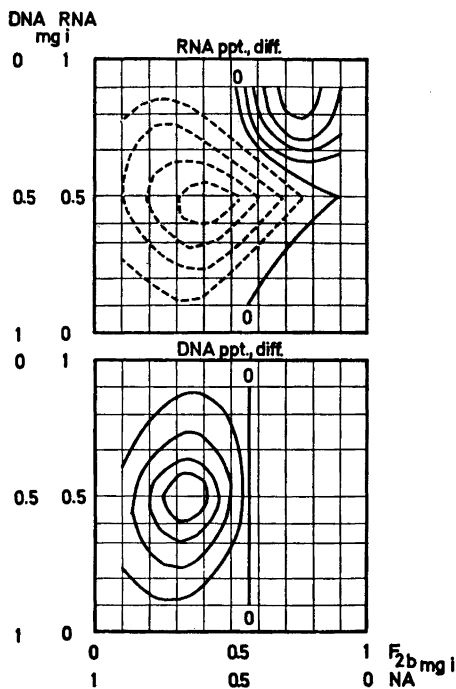


Fig. 2. Three dimensional graphs illustrating the mutual dependence of RNA and DNA precipitation obtained by combining the results from all test series of varied RNA, DNA, and F_{2b} ratios. One is exemplified in Fig. 1. Initial (i) RNA:DNA gradient, vertical axis. Initial (i) histone:nucleic acid gradient, horizontal axis. For F_{2b} the found and expected precipitation was the same all over the measured area. For RNA and DNA iso-precipitation lines are drawn for found minus expected amounts of precipitate, as defined in Fig. 1. The actual measuring points are the cross sections of the grid. The evenly spaced iso-precipitation lines are obtained though interpolation. Each step in the spacing equals +0.02 mg, unbroken lines, or -0.02 mg, dotted lines. The 0-line represents found = expected.

Below the histone:nucleic acid equivalence point of about 1.3, F_{2b} :nucleic acid = 0.57:0.43, a surplus of DNA precipitates and corresponding amounts of RNA stay in solution. Below this point, histone represents a limiting factor because all histone added was precipitated and thus none was available for further nucleic acid precipitation. Here the precipitation capacity of RNA and DNA separately is very much the same. However, as evident from Fig. 2, combined DNA and RNA compete for the limited amount of histone. On an average, DNA is precipitated one third more than expected and RNA one third less. Thus the average competitive capacity of DNA can be said to be twice that of RNA.

Above the histone:nucleic acid equivalence point, histone exists in excess and little competition should be anticipated. Accordingly, DNA was here completely precipitated independently of the presence of any amount RNA, Fig. 2. On the other hand, at initial RNA:DNA ratios above 1, a distinct precipitation maximum for RNA appears, Fig. 2. No supplementary histone was precipitated: as mentioned above, histone precipitated independently of the varied RNA:DNA ratio. One must therefore postulate a reaction formula of the type: DNA-histone + supplementary RNA \rightarrow DNA-RNA-histone, *i.e.* the formation of a mixed nucleoprotein complex.

Additional preliminary test series made with F_1 , F_{2a} , and F_3 demonstrate a similar but quantitatively somewhat different competition between DNA and RNA for these histones compared to the competition for F_{2b} . A mixed nucleoprotein complex formed only with F_3 .

The reported interactions between RNA and DNA at nucleohistone formation cannot simply be a question of preferential precipitation of the nucleic acids according to their molecular size. Below the equivalence point, separate ribosomal RNA and s-RNA are precipitated by histone quantitatively, just as yeast RNA. However, when competing with DNA, both the high molecular ribosomal RNA and the low molecular s-RNA showed a markedly higher competitive capacity than did the yeast RNA of intermediate size. When

competing with yeast RNA in equal proportions, single stranded DNA is completely dominant so that the nucleohistone precipitate contains only DNA. The observation that supplementary RNA can bind to DNA-histone shows that the rise in histone:DNA ratio of nucleoproteins earlier obtained¹ actually involves free binding sites for molecules large as RNA. In fact the high molecular ribosomal RNA had a greater ability to complex with DNA-histone than the yeast RNA. The exploitation seems to be specific. As mentioned above, yeast RNA complexed only with DNA- F_{2b} and DNA- F_3 , and only little RNA attached to single stranded DNA- F_{2b} .

An interpretation of the results from these model experiments can to some extent be given a cell physiological content. The fact that RNA competes with DNA for histone in an orderly manner underlines that RNA acting as a histone acceptor could contribute to at least part of the mechanism for gene activation.³ Further, the complexing of RNA with DNA-histone which seems to appear in these model experiments may correspond to the strong binding of RNA to histone and DNA found especially in rapidly dividing cells.⁴⁻⁶ This complex formation might imply gene or chromatin inactivation.

This investigation was facilitated by grants from the *Royal Physiographical Society*. Valuable technical aid was given by Miss Brita Nilsson.

1. Agrell, I. P. S. *Biochim. Biophys. Acta* **186** (1969) 226.
2. Kirby, K. S. *Biochem. J.* **96** (1965) 266.
3. Agrell, I. P. S. and Molander, L. *Exptl. Cell. Res.* **57** (1969) 104.
4. Agrell, I. P. S. *Pathol. Biol. Semaine Hop.* **9** (1961) 284.
5. Agrell, I. P. S. and Bergquist, H. A. *Comp. Biochem. Physiol.* **22** (1967) 189.
6. Huang, R. and Bonner, J. *Proc. Natl. Acad. Sci. US* **54** (1965) 960.

Received March 4, 1970.