constitutivity of the galactose operon; i.e., $K^-[Gal^c]$ represents the constitutivity of the synthesis of transferase and epimerase in galactokinaseless mutants. PEP stands for phosphoenolpyruvate.

* This work was supported by grants from the National Institutes of Health (AM 05639-05, AM 05507-05), and the National Science Foundation (GB-3646), and by the Joseph P. Kennedy, Jr., Foundation and the Wellcome Trust. One of the authors (H. C. W.) was supported by a fellowship from the Helen Hay Whitney Foundation.

[1] Kalckar, H. M., H. de Robichon-Szulmajster, and K. Kurahashi, *Proc. Intern. Symp. Enzyme Chem., Tokyo Kyoto, 1957* (1958), p. 52.

[2] Horecker, B. L., J. Thomas, and J. Monod, *J. Biol. Chem.*, **235**, 1580 (1960).

[3] Buttin, G., *J. Mol. Biol.*, **7**, 164 (1963).

[4] *Ibid.*, p. 183.

[5] Sundararajan, T. A., A. M. C. Rapin, and H. M. Kalckar, these PROCEEDINGS, **48**, 2187 (1962).

[6] Fukasawa, T., K. Jokura, and K. Kurahashi, *Biochim. Biophys. Acta*, **74**, 608 (1963).

[7] Kalckar, H. M., K. Kurahashi, and E. Jordan, these PROCEEDINGS, **45**, 1776 (1959).

[8] Kalckar, H. M., and T. A. Sundararajan, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 26 (1961), p. 227.

[9] Jordan, E., M. B. Yarmolinsky, and H. M. Kalckar, these PROCEEDINGS, **48**, 32 (1962).

[10] Wu, H. C. P., unpublished data.

[11] Shedlovsky, A., and S. Brenner, these PROCEEDINGS, **50**, 300 (1963).

[12] Hattman, S., and T. Fukasawa, **50**, 297 (1963).

[13] Hartman, P. E., *Carnegie Inst. Wash. Publ. 612* (1956), p. 35.

[14] Ebisuzaki, K., unpublished.

[15] Layne, E., in *Methods in Enzymology*, ed. S. P. Colowick and N. O. Kaplan (New York: Academic Press, 1957), vol. 3, p. 447.

[16] Novick, A., and M. Weiner, these PROCEEDINGS, **43**, 553 (1957).

[17] Lin, E. C. C., S. A. Lerner, and S. E. Jorgensen, *Biochim. Biophys. Acta*, **60**, 422 (1962).

[18] Ames, B. N., and R. G. Martin, *Ann. Rev. Biochem.*, **33**, 243 (1964).

[19] Rotman, B., *Biochim. Biophys. Acta*, **32**, 599 (1959).

[20] Rotman, B., personal communications.

[21] Schlesinger, S., P. Scotto, and B. Magasanik, *J. Biol. Chem.*, **240**, 4331 (1965).

[22] Schlesinger, S., and B. Magasanik, *J. Biol. Chem.*, **240**, 4325 (1965).

[23] Jordan, E., and M. B. Yarmolinsky, *J. Gen. Microbiol.*, **30**, 357 (1963).

---

*ALTERED REPRESSION OF SOME ENZYMES OF SULFUR UTILIZATION IN A TEMPERATURE-CONDITIONAL LETHAL MUTANT OF NEUROSPORA**

BY ROBERT L. METZENBERG AND JUDITH W. PARSON

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY,
UNIVERSITY OF WISCONSIN MEDICAL SCHOOL, MADISON

Communicated by James F. Crow, January 31, 1966

Temperature-conditional lethal mutants of microorganisms have long been known,[1] but only rather recently has evidence been gained about the metabolic nature of such lesions.[2–7] Previous work has shown that an ethionine-resistant, temperature-sensitive irreparable mutant (*r-eth-1*) of *Neurospora crassa* is characterized by a loss of control over methionine synthesis;[8, 9] it was inferred, but not proved, that the defect in *r-eth-1* involves an alteration of repression, rather than of end-product inhibition. The data presented below show that in *r-eth-1*

the repressibility of sulfite reductase, aryl sulfatase, and choline sulfatase by methionine is lost or greatly reduced. In the case of the two sulfatases, a separate and distinct repression system must also be lifted before enzyme synthesis can occur. The latter system appears to employ a precursor of cysteine as a corepressor, and is not affected by the *r-eth-1* mutation.

Materials and Methods.—$Na_2S^{35}O_3$ and $H_2S^{35}O_4$ were purchased from the Commissariat à L'Énergie Atomique (France), and Oak Ridge National Laboratory, respectively. The potassium salt of *p*-nitrophenyl sulfuric acid and DL + *allo*-cystathionine were obtained from the Sigma Chemical Co. Pyridoxal phosphate and 5,5′-dithio-*bis*-(2-nitrobenzoic acid) were purchased from Calbiochem and the Aldrich Chemical Co., respectively.

Origin, growth, and harvesting of strains: The origin of *wild-type* and *r-eth-1* strains has been described.[7, 8] The cysteine and methionine-requiring auxotrophs were obtained from the Fungal Genetics Stock Center, Dartmouth College, and were crossed to *r-eth-1* by standard techniques. In each case, temperature-sensitive, auxotrophic recombinants were readily isolated either from random spores or by tetrad analysis. The strains were grown in liquid culture with agitation at 25° in the salts medium of Fries[10] with sucrose (1.5%) as the carbon source, and variously supplemented as described in the text. The flasks were inoculated with conidia to a concentration of roughly 5×10^4/ml. The mycelia were harvested after 44–72 hr, depending on the growth rate of the strain employed. Senescent cultures gave low (and erratic) enzyme levels, and their use was avoided.

Assay of sulfite reductase: Mycelia were washed, homogenized, and the resulting enzyme preparations were incubated with substrates and cofactors exactly as described by Leinweber and Monty[11] with the exception that S^{35}-sodium sulfite (*ca.* 0.25 μc/μmole) was employed. The reaction was terminated by freezing the tubes in powdered dry ice. Carrier Na_2S and Na_2SO_3 (2 μmoles and 18 μmoles, respectively) were added, followed by 1 ml of 0.5 *M* $CdCl_2$ in 2.24 *M* acetic acid. The tubes were thawed, the CdS was collected on Millipore filters, and washed with cold 2.24 *M* acetic acid containing freshly dissolved carrier Na_2SO_3 (0.1 *M*). The filters were dried, glued to planchets, and radioactivity was measured in a gas flow counter. For a standard, an aliquot of the radioactive substrate in dilute NaOH was assayed on a copper planchet, and the data were corrected for the decreased backscatter of the filters as compared with the copper. A somewhat analogous analytical method employing CdS precipitation has been described.[12] The production of S^{35}-sulfide by undialyzed enzyme was reduced about tenfold in various experiments by the omission of either flavin adenine dinucleotide or nicotinamide adenine dinucleotide phosphate. Dialysis, Sephadex treatment, or ammonium sulfate precipitation of the crude preparations did not further decrease this residual activity.

Assay of choline sulfatase: Mycelia grown as described above were washed with water, ground with sand in the presence of 0.05 *M* Tris-HCl buffer (pH 8.1), and the homogenates were centrifuged for 20 min at 30,000 × *g*. An aliquot (0.2 ml) of the supernatant solution, containing 0.5–1.0 mg of protein, was incubated for 60 min at 37° with 2.5 μmoles (0.05 μc) of synthetic S^{35}-choline sulfate[13] and 5 μmoles of Tris-HCl buffer, pH 7.5, in a final volume of 0.5 ml. The reaction was stopped by adding 2 ml of 10% trichloroacetic acid containing $MgSO_4$ carrier (0.001 *M*), followed by 0.05 ml of barium acetate (1.0 *M*). The $BaS^{35}O_4$ was collected on Millipore filters, washed twice with 5 ml of water, and assayed as described above. A sample of the substrate, hydrolyzed in a sealed tube for 18 hr at 120° and carried through the above procedure, served as a standard, and all determinations were corrected by means of a nonenzymatic blank.

Assay of aryl sulfatase: Crude *Neurospora* supernatant enzyme, prepared as described above, was incubated at 37° with 5 μmoles of potassium *p*-nitrophenyl sulfate and 250 μmoles of Tris-HCl (pH 8.1) in a final volume of 1.0 ml. Kinetic studies showed that the K_m for *p*-nitrophenyl sulfate is 2.0×10^{-3} *M*, i.e., that 71% of the maximum velocity is being measured in the above assay system. The incubations were terminated after 10 or 60 min, according to the activity of the sample, by the addition of 0.5 ml of 2 *N* NaOH, and *p*-nitrophenol liberation was measured at 405 mμ. The readings were corrected for the slight endogenous absorbancy of the substrate and turbidity of denatured protein by appropriate blanks from which enzyme and substrate, respectively, had been omitted. In certain cases, in which extremely low activities were to be measured, it was profitable to add as much as 1 mg of *Neurospora* protein to the incubation system

and, after stopping the reaction, to remove denatured protein completely. This was accomplished by adding an equal volume of 1,1,2,2-tetrachloroethane, stirring the mixture vigorously, and separating the phases by centrifugation. The aqueous layer, containing the nitrophenol, was carefully removed without disturbing the denatured protein at the interface.

Parenthetically, it may be noted that aryl sulfatase and choline sulfatase are distinct enzymes in *Neurospora*, as they apparently are in *Pseudomonas aeruginosa*.[14] The evidence, to be presented elsewhere, includes differential heat inactivation as well as facile separation of the two activities by chromatography on DEAE-cellulose.

γ-Cystathionase was measured on crude extracts as described by Flavin.[15] Protein analyses were performed by the method of Lowry *et al.*,[16] using bovine serum albumin as a standard. Specific activities of all enzymes are reported as mμmoles/min/mg of protein, referable to the above assay conditions.

Results and Discussion.—Control of sulfite reductase: In Table 1 it can be seen that the presence of high concentrations of methionine during growth represses the formation of sulfite reductase in *wild-type Neurospora*, but has relatively little effect on *r-eth-1*. Methionine added *in vitro* to the incubation system was without effect, even at a concentration of 5×10^{-3} *M*. Cysteine at a concentration of 5×10^{-4} *M* inhibited the sulfite reductase of both *wild type* and *r-eth-1* by 23 per cent, but was not measurably inhibitory on either preparation at 5×10^{-5} *M*. Hence, changes in the repression of sulfite reductase in *r-eth-1* are consistent with the overproduction of methionine by this strain, whereas no alteration of end-product inhibition is evident.

Repression of aryl sulfatase and choline sulfatase: Fries' minimal medium contains a relatively high concentration of inorganic sulfate (2×10^{-3} *M* $MgSO_4$). When either *wild type* or *r-eth-1* is grown on this medium, little or no aryl sulfatase is produced. As seen in Table 2, limitation of inorganic sulfate causes derepression of the synthesis of aryl sulfatase in *wild type*, a finding which is consistent with the

TABLE 1

REPRESSION OF SULFITE REDUCTASE BY METHIONINE

L-methionine in growth medium (mM)	Sulfite Reductase, Sp. Activity	
	Wild type	*r-eth-1*
0.00	1.8	2.0
0.10	1.2	1.3
0.25	0.9	1.5
1.00	0.4	1.3
2.5	0.2	1.0
5.0	0.2	1.2

The strains were grown, and enzyme assayed as described in the text.

TABLE 2

REPRESSION OF ARYL SULFATASE BY EXOGENOUS INORGANIC SULFATE

Strain	SO_4^{-2} in medium (mM)	L-methionine in medium (mM)	Aryl sulfatase, sp. activity
Wild type	0.05	—	58
"	0.1	—	44
"	0.2	—	27
"	0.5	—	3.6
"	1.0	—	<0.2
"	2.0	—	<0.2
"	5.0	—	<0.2
"	2.0	0.25	<0.2
cys-5	—	0.25	51
"	5.0	0.25	44

The $MgSO_4$ normally present in Fries' medium was replaced by its molar equivalent of $MgCl_2$, and the usual trace metals were provided as chloride salts. K_2SO_4 was added to give the desired sulfate concentrations.

behavior of this enzyme in *Aerobacter*,[17, 18] and in various fungi.[19] Yet inorganic sulfate does not in itself promote repression. Ragland[20] has reported, and we have confirmed, that the mutant *cys-5* lacks ATP-sulfurylase, the first enzyme in the pathway of sulfate anabolism. From Table 2, it is clear that *cys-5* grown at a low methionine concentration is not appreciably repressed by exogenous sulfate. Presumably, this is due to the failure of this strain to activate sulfate and subsequently reduce it to a corepressor.

Since the sulfite reductase system had been found to be repressible by methionine, it was of interest to see whether production of the sulfatases was also normally under methionine control, and if so, whether the latter control is lost in the presence of the *r-eth-1* gene. Table 3 shows the levels of aryl and choline sulfatases in various strains which were grown at "low" (0.25 mM) and "high" (5.0 mM) methionine. Several facts are immediately apparent. (1) Methionine at high concentration completely represses the production of aryl and choline sulfatase by *cys-5* unless *r-eth-1* is also present in the genome. (2) Qualitatively, the same sort of behavior is seen in the three nonallelic sulfite reductase mutants. Quantitatively, the picture is complicated by the fact that in these mutants, *r-eth-1* confers only partial protection against methionine repression of aryl sulfatase; in addition, the aryl sulfatase of *cys-10* itself is incompletely repressible by methionine. (3) No sulfatase of either type could be unequivocally detected in *cys-3*, which has been reported to lack the sulfate permease.[21]

The *cys-5, r-eth-1* strain, which is insensitive to inorganic sulfate and to high methionine concentrations, can nevertheless be repressed under suitable conditions; Table 4 shows that inorganic thiosulfate and, to a lesser extent, sulfite are effective in this respect, whereas cystine is ineffective. Harada and Spencer[17] have found that thiosulfate is particularly effective in repressing the aryl sulfatase of *Aerobacter aerogenes*, but suggest that the active compound may be cysteine, whereas Rammler *et al.*,[18] working with the same enzyme and organism, suggest that the corepressor is closely related to inorganic sulfate itself. In *Neurospora*, thiosulfate is apparently not on the main pathway of sulfur reduction, but gives rise to sulfide and sulfite by a reductive cleavage.[11] Hence, growth in the presence

TABLE 3

REPRESSION OF CHOLINE AND ARYL SULFATASES BY EXOGENOUS METHIONINE

Lesion causing auxotrophy	Strain	Aryl Sulfatase, Sp. Activity L-Methionine, (mM) 0.25	Aryl Sulfatase, Sp. Activity L-Methionine, (mM) 5.0	Choline Sulfatase, Sp. Activity L-Methionine, (mM) 0.25	Choline Sulfatase, Sp. Activity L-Methionine, (mM) 5.0
None	*Wild type*	<0.2	<0.2	0.05	<0.02
	r-eth-1	<0.2	<0.2	<0.02	<0.02
ATP sulfurylase	*cys-5*	52	<0.2	1.13	<0.32
	cys-5, r-eth-1	77	60	0.88	0.31
Sulfite reductase	*cys-4*	80	<0.2	0.97	<0.02
	cys-4, r-eth-1	38	3.5	0.18	0.03
	cys-2	10.5	<0.2	0.08	<0.02
	cys-2, r-eth-1	41	9.4	0.12	0.03
	cys-10	42	2.4	0.17	0.02
	cys-10, r-eth-1	50	5.2	0.42	0.04
Sulfate permease	*cys-3*	<0.2	<0.2	<0.02	<0.02
	*cys-3, r-eth-1**	—	—	—	—

The strains were grown on ordinary Fries' medium supplemented with methionine as described above.
* This double mutant was isolated by tetrad analysis and its genotype confirmed by outcrossing. Unfortunately it grows only extremely slowly, especially on liquid media, and it has not been possible to make any meaningful tests with it. Attempts to isolate more vigorous strains of *cys-3, r-eth-1* have merely yielded more examples with the same phenotype.

TABLE 4

REPRESSION OF ARYL SULFATASE IN *cys-5, r-eth-1* BY VARIOUS SULFUR COMPOUNDS

Metabolite added	Concentration (mM)	Aryl sulfatase, sp. activity
None	—	46
$Na_2S_2O_3$	1.0	15
	2.0	10.5
	5.0	1.3
Na_2SO_3	1.0	42
	2.0	24
	5.0	4.9
L-cystine	0.5	47
	1.0	54
	2.5	67

All flasks were charged with Fries' medium supplemented with L-methionine (5.0 mM) and additionally as described above. The $Na_2S_2O_3$ and Na_2SO_3 were freshly prepared solutions which were filter-sterilized and added to the flasks immediately before inoculation.

of thiosulfate furnishes the cells with a constant trickle of both of these intermediates. Just as the derepression of *cys-5* in the presence of sulfate shows that sulfate is merely a precursor of a corepressor, the derepression of the three sulfite reductaseless mutants argues that sulfite is likewise a mere precursor. Yet the corepressor appears to be prior to cyst(e)ine in the pathway and therefore it seems likely that sulfide or a closely related metabolite is a corepressor *per se*. Unfortunately, it has not been practical to furnish sulfide to *Neurospora* except via thiosulfate, as exogenous sulfide is volatile, auto-oxidizable, and quite strongly inhibitory to growth. For this reason, it has not yet been possible to pinpoint the corepressor on the metabolic map with any great degree of confidence.

An obvious prediction of the above model is that methionine deprivation will not in itself effect derepression of aryl sulfatase synthesis, and that auxotrophs blocked between cyst(e)ine and methionine will be repressed even on limiting methionine, as long as the cultures are replete with respect to sulfate. Table 5 shows that methionine auxotrophs and their double mutants with *r-eth-1* produce much less aryl sulfatase than do strains which lack enzyme for cyst(e)ine biosynthesis, and many form no detectable aryl sulfatase at all. Nevertheless, the lack of absolute repression in several strains, particularly *me-8*, suggests that there may be some complex interactions between repression by methionine and by sulfide and its congeners.

TABLE 5

SPECIFIC ACTIVITY OF ARYL SULFATASE IN METHIONINE-REQUIRING AUXOTROPHS AND IN DOUBLE MUTANTS WITH *r-eth-1*

Auxotrophic strain	Allele at *r-eth-1* Locus: *Wild type*	Allele at *r-eth-1* Locus: *r-eth-1*
me-1	0.3	0.2
me-2	0.9	2.5
me-3	<0.2	<0.2
me-5	<0.2	<0.2
me-6	<0.2	<0.2
me-7	0.2	1.3
me-8	4.6	*
me-9	0.7	*
me-UT-27	<0.2	<0.2
me-UT-70	<0.2	<0.2
me-UT-112	0.8	0.5

All flasks were charged with Fries' medium supplemented with a low concentration of L-methionine (0.25 mM).

* Strains *me-8,r-eth-1* and *me-9,r-eth-1* were isolated, but grew too slowly on limiting methionine to be used here.

It seems clear that the "early" or "sulfide-related" corepressor does not function via conversion to methionine. What evidence can be brought to support a symmetrical argument, viz., that there is a "late" or "methionine-related" corepressor which functions by means other than conversion to "early" corepressor? If one holds that no "late" corepressor exists, two successive and rather *ad hoc* conclusions are necessary: (1) cyst(e)ine produced endogenously from methionine gives rise to "early" corepressor, but exogenous cyst(e)ine fails to do so; (2) in *r-eth-1*, methionine cannot be converted to cyst(e)ine. The crucial enzyme in this conversion would presumably be γ-cystathionase. We have measured this enzyme in *wild type* and *r-eth-1;* the results, presented in Table 6, show that there is no dramatic

TABLE 6

γ-CYSTATHIONASE ACTIVITY IN *wild type* AND *r-eth-1*

Strain	L-methionine in medium (mM)	γ-Cystathionase, sp. activity
Wild type	—	4.8
	2.5	15
r-eth-1	—	4.4
	2.5	7.1

The strains were grown in Fries' medium with or without added methionine. The data were corrected (as described by Flavin[18]) for reduction of ArSSAr by protein itself and by traces of free mercaptan in the substrate.

difference between the two strains, either in the presence or absence of methionine. Hence, it seems likely that there exist two independently sufficient corepressors of aryl sulfatase synthesis in *Neurospora*, an "early" one related to sulfide, and a "late" one related to methionine. The latter is missing or nonfunctional in *r-eth-1* and is involved in the control of the two sulfatases, of sulfite reductase, and perhaps of other enzymes as well. The existence of dual repression systems is known in *Escherichia coli*, where catabolite repression of β-galactosidase synthesis occurs even in "constitutive" strains,[22] and is mediated by a distinct genetic system.[23]

Finally, the "anomalous" mutant *cys-3* must be considered. This strain produces no detectable aryl sulfatase under the previously specified conditions, nor when grown on limiting methionine or limiting sulfite in the absence of sulfate. Nine additional reisolates from an outcross of *cys-3* to *wild type* likewise produced no aryl sulfatase. One of these reisolates gave a value for choline sulfatase which was near or at the borderline of detection; the others were negative. Hussey *et al.*[24] have reported on a mutant of *Aspergillus nidulans* which lacks sulfate permease. This mutant readily produces choline sulfatase and, unlike *cys-3* of *Neurospora*, will grow on choline sulfate as a sole sulfur source. The findings with *cys-3* can be interpreted in several ways that are consistent with current theories. Conceivably, sulfate permease, aryl sulfatase, and choline sulfatase have a polypeptide chain in common, and this chain is defective in *cys-3*. Alternatively, these three activities might be specified by genes in a single operon, in which case *cys-3* might be analogous to an *O*° or extreme polarity mutation.[25] Finally, the structural genes for these activities might be widely scattered, but nevertheless be under control of the same repressor; *cys-3* could then be viewed as a superrepressor mutation,[26] in which (in this case) an aporepressor of an anabolic pathway has been altered so that it is active in the absence of corepressor. These alternatives will be subject to test when mutants with qualitatively altered sulfatases or sulfate permease become available for genetic mapping.

The following alleles were used: *cys-2*, 80702; *cys-3*, P-22; *cys-4*, P-1; *cys-5*, 85518; *cys-10*, formerly called *me-4* (Murray[27]) 39816; *me-1*, 38706; *me-2*, 48004; *me-3*, 36104; *me-5*, 9666; *me-6*, 35809; *me-7*, 4894; *me-8*, P-53; *me-9*, C-124. *me-UT-27*, *me-UT-70*, and *me-UT-112* are allele numbers to which locus numbers have not yet been assigned. Since the preparation of this paper, new terminology for resistant mutants has been proposed.[28] In the new nomenclature, *r-eth-1* would be identified as *eth-1*^{r}.

* This work was supported in part by a U.S. Public Health Service grant (GM-08995-04).

[1] Horowitz, N. H., and U. Leupold, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 26 (1951), p. 65.
[2] Eidlic, L., and F. C. Neidhardt, *J. Bacteriol.*, **89**, 706 (1965).
[3] Eidlic, L., and F. C. Neidhardt, these PROCEEDINGS, **53**, 539 (1965).
[4] Yaniv, M., M. Kohiyama, F. Jacob, and F. Gros, *Compt. Rend.*, **260**, 6734 (1965).
[5] Wiberg, J. S., and J. M. Buchanan, these PROCEEDINGS, **51**, 421 (1964).
[6] Edgar, R. S., and R. H. Epstein, *Sci. Am.*, **212**, 70 (1965).
[7] Metzenberg, R. L., M. S. Kappy, and J. W. Parson, *Science*, **145**, 1434 (1964).
[8] Kappy, M. S., and R. L. Metzenberg, *Biochim. Biophys. Acta*, **107**, 425 (1965).
[9] Galsworthy, S. B., and R. L. Metzenberg, *Biochemistry*, **4**, 1183 (1965).
[10] Fries, N., quoted in Beadle, G. W., and E. L. Tatum, *Am. J. Botany*, **32**, 678 (1945).
[11] Leinweber, F. J., and K. J. Monty, *J. Biol. Chem.*, **240**, 782 (1965).
[12] Segel, I. H., and M. J. Johnson, *Arch. Biochem. Biophys.*, **103**, 216 (1963).
[13] Stevens, C. M., and P. Vohra, *J. Am. Chem. Soc.*, **77**, 4935 (1955).
[14] Harada, T., *Biochim. Biophys. Acta*, **81**, 193 (1964).
[15] Flavin, M., *J. Biol. Chem.*, **237**, 768 (1962).
[16] Lowry, O., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[17] Harada, T., and B. Spencer, *Biochem. J.*, **93**, 373 (1964).
[18] Rammler, D. H., C. Grado, and L. R. Fowler, *Biochemistry*, **3**, 224 (1964).
[19] Harada, T., and B. Spencer, *Biochem. J.*, **82**, 148 (1962).
[20] Ragland, J. B., *Arch. Biochem. Biophys.*, **84**, 541 (1959).
[21] Leinweber, F. J., personal communication cited by Murray.[27]
[22] Mandelstam, J., *Biochem. J.*, **82**, 489 (1962).
[23] Loomis, W. F., Jr., and B. Magasanik, *Biochem. Biophys. Res. Commun.*, **20**, 230 (1965).
[24] Hussey, C., R. A. Orsi, J. Scott, and B. Spencer, *Nature*, **207**, 632 (1965).
[25] Beckwith, J. R., *Biochim. Biophys. Acta*, **76**, 162 (1963).
[26] Willson, C., D. Perrin, M. Cohn, F. Jacob, and J. Monod, *J. Mol. Biol.*, **8**, 582 (1964).
[27] Murray, N., *Genetics*, **52**, 801 (1965).
[28] Barratt, R. W., and D. D. Perkins, *Neurospora Newsletter*, **8**, 23 (1965).

---

*PARTICIPATION OF A DNA-RNA HYBRID COMPLEX IN IN VIVO GENETIC TRANSCRIPTION**

BY MARIE N. HAYASHI AND MASAKI HAYASHI

DEPARTMENT OF BIOLOGY, UNIVERSITY OF CALIFORNIA, SAN DIEGO (LA JOLLA)

Communicated by S. Spiegelman, January 17, 1966

In a previous report[1] from this laboratory it was shown that a DNA-RNA complex functions as an intermediate in genetic transcription *in vitro*. These studies employed a purified "replicative form"[2] (RF-DNA) of ΦX-174 as a template for the DNA-dependent RNA polymerase isolated from *E. coli* (C-122). The principal characteristics of the DNA-RNA complex may be summarized as follows: (1) the two nucleic acids are combined in a structure resistant to phenol, chloroform, de-