

TRANSDUCTIONAL ANALYSIS OF MONOPHASIC NATURE OF STRAIN SW1061.

Report by Tetsuo Iino

(July 6, 1956)

EXPERIMENTS.

1). Alternative changes between 1.2 and Fla⁻ in SW1061 culture.

The original stab culture of SW1061 was streaked on EMB-gal plate and a colony, which has 1.2 antigen, was transferred to penassay broth medium. After 2 days at 37C, the culture was streaked again on EMB-gal plate, and antigen types of the colonies grown in the plate were tested by slide agglutination. 8 among 20 colonies tested showed 1.2 reaction, whereas the remained 12 colonies did not agglutinate by either anti-i or anti-1.2 serum, or even by polyvalent serum. Single colonies of 1.2 antigen type and nonagglutination type (-) were isolated to penassay broth in each, and, after 2 day culture, they were streaked again on EMB-gal plate and also plated with mot agar. The antigen type and the motility of the colonies grown were listed in table 1: 1.2 and (-), and motile and non-motile appeared mixed.

Correspondence of 1.2 types to motile^s and (-) to non-motile^s were confirmed by testing the antigen type of motile and non-motile colonies after isolating to penassay broth and by testing the motility of 1.2 and (-) on mot-agar plates.

These results are repeatable on single colony isolates, and indicate that monophasic SW1061-1.2 strain produces frequently non-motile H-negative (Fla⁻) subclones, which in turn revert frequently to motile cells with 1.2 antigen.

2). Transduction of H from SW803 (b:enx) to SW1061 (1.2).

Penassay broth cultures of SW1061 (mixture of 43% 1.2-type cells and 57% (-) cells) were mixed with the lysate of phase 2 culture of SW803 and brushed on mot-agar plate supplemented anti-1.2 serum. 38 swarms grown were isolated to penassay broth media and antigen types were examined. 24 among 38 showed "b" type, 13 "enx", and remained one "i" (Table 2).

They were selected by mot-stab containing corresponding antiserum in order to see alternative phase. Of 24 b cultures, 23 produced swarm^s on anti-b mot-agar media. The other one has not produced swarm even on repeated selection by anti-b mot-agar stab^s. Swarms of alternative phase didn't develop from 13 enx culture^s at all. The i-phase culture grew swarms which showed 1.2 type. i:1.2 are the alternative antigens of TM2, the ancestor of SW1061, so that last type may appear either by transduction or by reversion, or by both. If reversion is involved in the development of type IV (i:1.2), ^{this} i-type must also appear from SW1061 without transduction. This was proved by selecting SW1061 by anti-1.2 in mot stabs. Among 20 anti-1.2 mot agar stabs, 5 produced swarms which were agglutinated by anti-i. They were all diphasic i:1.2 in successive subcultures. Thus, monophasic strain SW1061-1.2 changes to diphasic either by reversion or by transduction of H₁ but not by transduction of H₂. with one apparent exceptional case in b-transduced monophasic clone.

Transduction from TM2 (i:1.2) to the b-transduced monophasic clone (type II in table 4) showed that this is also the ^a case to which above rule is applicable. The mixture of the penassay broth culture of type II clone with the lysate of phase 2 culture of TM2 was brushed on mot-agar plates supplemented anti-b serum as selective agent. 20 swarms developed were isolated and antigen types and alternative phases were tested by the same method as the foregoing experiment. The results, summarized in table 3, indicates that b is replaced not by i but 1.2, that is H^b in type II clone is not H₁ loci but H₂.

10 clones were sampled from b:(1.2) and enx-monophasic transductions, and also from original SW1061-1.2 monophasic cultures as controls. They were cultured in penassay broth media in parallel for two days, and plated with mot-agar. The numbers of non-motile and motile colonies grown in these plates were listed in table 4. Both original monophasic 1.2 and enx-transduced monophasics produced non-motile subclones, which in turn produced motile cells with the same antigen as before, in high frequencies, whereas b clones which changed from monophasic to diphasic, lost the potency to produce non motile subclones in high frequencies. Specially i:1.2 clones, both obtained on the course of transduction and anti-1.2 selection, lost the potency to produce non-motile subclones, ~~in high frequencies~~ whereas monophasic b clone has still continued to produce non-motile subclones. One of the non-motile subclones obtained from b:(1.2) plate (see table 3) is very stable and reversion to motile was not observed. Another one is reversible and produces motile cells frequently. However, the motile cells produced are diphasic b:1.2 and do not produce non-motile subclones differed from the motiles obtained from non-motile subclones of monophasic SW1061(1.2). These results are summarized in brief that the change from monophasic to diphasic couples with the loss of the potency to change between motile and non-motile.

These results may well be explained by assuming that, in SW1061, H₁ is inactive, by its own structural change or by ~~the~~ suppressive action of the factor closely linked to it, remained its specificity to produce i antigen unexpressed, whereas H₂ changes its state as in usual diphasic strain, and when H₂ is active, phase 2 (1.2) antigen is produced but when H₂ changes to inactive, that is both H₁ and H₂ are inactive, H-antigen cannot be produced and the cell becomes to Fla⁻. Two non-motile colonies obtained from b(1.2) plate may be Fla⁻ mutants different from the inactive H₁.

3). Transductions from SW1061 to SW666.

It has been already known that Fla₆₆₆ is transduced from Fla⁺ strains to SW666 (b, monophasic Fla⁻) linked with H₁ at about 10% frequency. If H₁ in SW1061 is inactive, as has been assumed in the previous chapter, linked transduction produces Fla⁻ (Fla₆₆₆⁺, H₁ inactive) and could not be recovered by the Fla⁺ selection on mot agar. So, SW1061 -x SW666 will not produce Fla⁺ transduction which is transduced phase 1 antigen simultaneously.

1 ml of overnight penassay broth culture of SW666 were divided into three parts and 1 ml of SW1061-lysate, TM2(phase 2)-lysate or saline were added to the each. After 15 minutes, they were centrifuged and concentrated cell suspensions were brushed on mot agar plates. Swarms developed from them were isolated to EMB-gal media and antigen types were tested by slide agglutination. The results were summarized in table 5. 8 among 64 (1.25%) Fla₆₆₆ transductions were linked with H₁ in TM2 -x SW666, whereas linked transduction was not found out in 77 Fla₆₆₆ transductions in SW1061 -x SW666, indicating the validity of the assumption.

DISCUSSION.

Monophasic nature of SW1061 is not caused by the stabilization of H_2 but inactivation of H_1 . The state change of H_2 in this strain occurs samely as in diphasic strains and expresses 1.2 type and Fla⁻ alternatively, which is in parallel with phase variation in diphasic strains.

Inactive H_1 can revert and express the same antigen as the original diphasic ancestor. This suggests inactive H_1 still holds its specificity on the antigen control as inactive H_2 does so. Whether the activation and the inactivation of H_1 is homologous phenomenon with the H_2 -state change, differed only in their frequencies, or different in nature is unknown. As far as has been tested, there is no significant difference between the frequency of the production of i:1.2 clone by reversion and of the course of transduction, but the possibility of the suppression by the adjacent factor may still have to be held until the more quantitative comparison of the frequencies is completed.

The present results indicate that either one of active H (H_1 or H_2) is enough for the production of H-antigen but inactivation of both losses the producibility of H-antigen, suggesting H_1 and H_2 control the parallel steps of flagellar formation followed to the common steps which are controlled by different Fla loci.

The process of the appearance of a b-type phase 2 monophasic strain from SW803 -x SW1061 may involve the replacement of $H_2^{1.2}$ by H^b . Whether the H^b produced differed from H_1^b or not and whether replacement are made between whole structure of each H locus or only a part which controls antigen type specificity, are unknown. The interesting point is that H^b in H_1 locus is stable compared to H_2^{enx} or $H_2^{1.2}$ whereas H^b in H_2 locus is unstable like as $H_2^{1.2}$. This suggests the unstability of H_2 is not relate with antigen type specificity but with locus specificity.

Table 1.

Types of cells in 2 day penassay broth culture of SW1061-1.2
or -(-) clone.

Antigen type of original clone	number of colonies				
	which show 1.2	antigen (-)	on mot-agar plates		
			swarm	halo	compact
1.2	5	5	43	7	59
(-)	4	6	11	10	59

Table 2.

Transduction of H from SW803 (b:aux) to SW1061 (1.2).

(Transductions were selected by anti 1.2 serum-mot-agar.)

Type	No. of swarms	Antigen type	Alternative antigen type
I	23	b	1.2
II	1	" b "	-
III	13	aux	-
IV	1	i	1.2

Table 3.

Transduction of H from TM2(phase 2 culture) to the b-
monophasic transduction clone (type II in Table 2).

No. of swarms	Antigen type	Alternative antigen type	Fla ⁻ subclone
7	i	" b "	hit produced
13	1.2	-	hit produced

SW 1161

SW 1162

Table 4.

Composition of motile and non-motile cells in 2day penassay broth cultures of type I and type III transduction clones.

Type	No. of clones	Total no. tested	Swarm	Halo	Compact
I. b:(1.2)	1	115	115	0	0
	2	142	141	0	1
	3	174	174	0	0
	4	263	262	0	1
	5	23	23	0	0
	6	126	126	0	0
	7	305	305	0	0
	8	117	117	0	0
	9	121	121	0	0
	10	225	225	0	0
	Total	1611	1609	0	2
III. enx	1	65	24	13	28
	2	163	79	14	70
	3	275	133	23	119
	4	183	62	38	83
	5	90	56	9	25
	6	136	40	22	74
	7	103	47	24	32
	8	152	68	23	61
	9	146	53	14	79
	Total	1313	562	180	571
SW1061 1.2 (control)	1	112	66	15	31
	2	179	90	11	78
	3	91	47	10	34
	4	41	12	8	21
	5	76	39	11	26
	6	43	22	9	12
	7	35	20	3	12
	8	331	134	32	165
	9	50	24	6	20
	10	48	19	5	24
	Total	11006	473	110	423

Table 5.

H-antigen types of Fla₆₆₆ transductions in SW1061 -x SW666 and in TM2 -x SW666.

Donor	Recipient	No. of swarms (Fla ⁺) recovered	Antigen types of Fla transduction	
			b	1
SW1061	SW666	77	77	0
TM2 phase2	SW666	64	56	8
	SW666	0	0	0