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Clonal Analysis in the Examination of Gene Function in *Drosophila*

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1. Introduction

Clonal analysis in *Drosophila* has been successfully used to address numerous biological questions of fundamental importance, including issues of cell lineage, fate determination, autonomy of gene action and pattern formation ***(1,2)***. Clonal analysis has been particularly useful for the study of genes that would be lethal in a homozygous mutant state; this approach also makes it possible to recover essential genes in mosaic screens ***(3)***.

Among the methods traditionally used by researchers to generate clones in *Drosophila*, the most frequent technique has been the induction of mitotic recombination through ionizing radiation such as X-rays ***(4–6)***. X-ray irradiation causes chromosomal breaks that can lead to the exchange of homologous chromosome arms; at mitosis, daughter cells may inherit a homozygous region distal to the point of recombination (*see* **Fig. 1**). Mitotic recombination events induced by X-rays take place at low frequencies, a factor that cripples the efficiency of most clonal analyses using this technique.

Use of the FLP–FRT yeast site-specific recombination system provides an efficient method for generating clones at high frequencies for phenotypic analysis and screening (*see* **Fig. 2**; ***[7–9]***). Strains have been constructed such that expression of the site-specific FLP recombinase can be driven by a heat-inducible promoter (*see* **Table 1**). Clones for almost any gene of the *Drosophila* genome can be produced once the gene of interest has been recombined onto specially engineered FRT-carrying chromosome arms (*see* **Tables 2** and **3**). And a sizable array of markers is available, facilitating the choice of a genetic marker appropriate for the tissue and developmental stage being studied (*see* **Tables 4–6**).

Protocols for using the FLP/FRT system to generate both somatic and germline clones are given below. Because some genes are not amenable to FLP/FRT clonal analysis, equivalent protocols for X-ray-induced clone production are also provided. Successful clone production for both protocols critically depends upon the timing of clone induction, as mitotic recombination can be induced only in cells that are actively dividing. For this reason, a timeline of cell divisions in specific tissues of the developing

From: *Methods in Molecular Biology, Vol. 137: Developmental Biology Protocols, Vol. III*
Edited by: R. S. Tuan and C. W. Lo

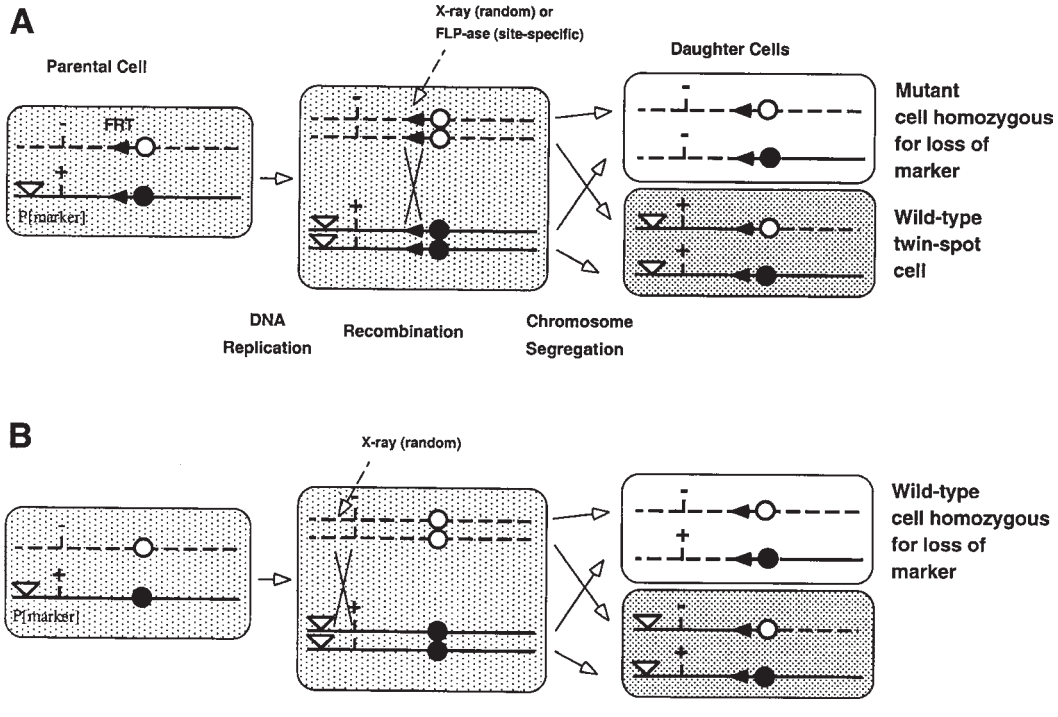

Fig. 1. **(A)** Use of the FLP–FRT system or X-rays to induce mitotic recombination and clone formation. Mutant clones are identifiable by concomitant loss of a marker gene. **(B)** Because X-rays induce recombination at random points along the chromosome, the marker gene must be located more proximal to the centromere than the mutation under study in X-ray induced clonal analysis. If the marker is more distal, some random X-ray events will generate marked wild-type clones (false positives). Because the action of FLP-ase is site-specific, proximity of the marker relative to the mutation is not important in FLP-FRT analysis.

fruit fly (*see* **Fig. 3**) is included to aid the researcher in designing successful clonal analyses.

2. Materials

Information for *Drosophila* strains is provided in **Tables 1–6**.

3. Methods

3.1. Induction of Somatic Clones by (a) FLP/FRT or (b) X-rays (see Note 1)

1. Set up crosses of the appropriate genotypes at 25°C (**Fig. 2**; *see* **Notes 2–4**).
2. Collect eggs for 12 h at 25°C.
3. Age eggs for 24 h (large adult clones) to 48 h (smaller, more frequent adult clones) at 25°C (*see* **Note 5**).

4a. Heat shock vials for 60 min in a 38°C water bath (*see* **Notes 6** and **7**).

4b. Place vials containing larvae close to X-ray source and expose to 1000R dose (*see* **Note 6**).

5. Return vials to 25°C for recovery.

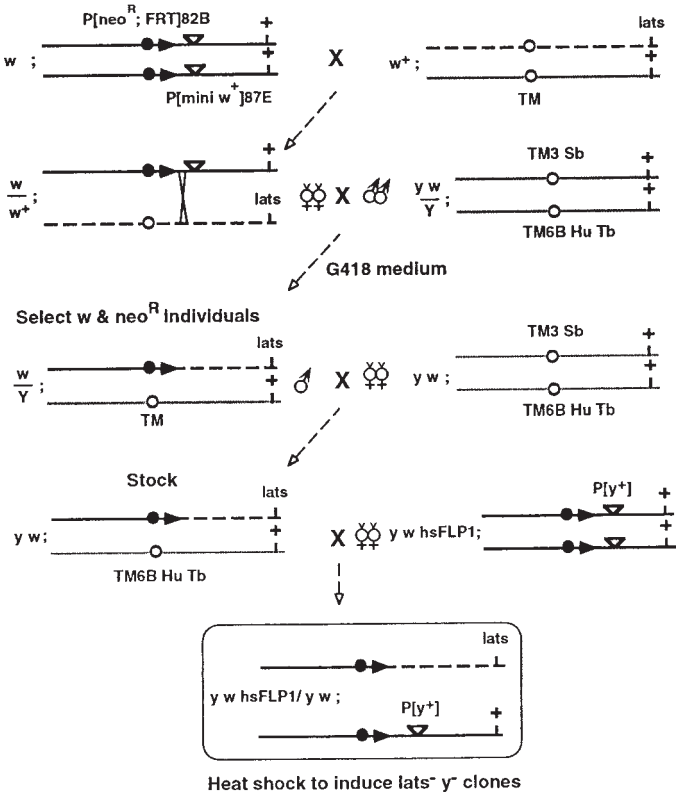

Fig. 2. An example scheme of crosses for recombining an allele of *lats* onto an FRT chromosome for FLP–FRT analysis.

Table 1
FLP Chromosomes

Chromosomes	Strains	Footnotes
X	*y w hsFLP1; Adv/CyO*	*a–c*
	y w hsFLP1; TM3, Sb/TM6B, Hu	*a–c*
	y hsFLP1; Bc; kar^{2} ry^{506}	*a,g*
	y w hsFLP122	*d*
	y w hsFLP122; TM3, ry^{RK} Sb/TM6B, Hu	*d,e*
	y w hsFLP^{12}; Sco/CyO	*a,f*
	y w hsFLP^{22}; CxD/TM3, Sb	*a,f*
	w; UAS-FLP	*c*
	yw; Ey-FLP	*i*
2	*y; hsFLP38 Bc/CyO; Ki kar^{2} ry^{506} Tb*	*a,g*
	pr pwn hsFLP38/CyO; Ki kar^{2} ry^{506}	*a,g*
	w; UAS-FLP	*c*
	yw; Ey-FLP	*i*
3	*hsFLP3, MKRS/TM6B*	*a,b,h*
	w; UAS-FLP	*c*

[a]Golic and Lindquist, 1989; [b]Xu and Rubin, 1993; [c]Xu, T., et al., unpublished; [d]Struhl and Basler, 1993; [e]Ito, N., et al., unpublished; [f]Chou and Perrimon, 1996; [g]Heitzler, P., unpublished; [h]Jan, Y. N., et al., unpublished; [i]Dickson, B., unpublished.

Table 2
FRT Elements

Chromosomes	Insertions	Code	Frequencies of recombination	Footnotes
X	*P[mini w+; FRT]14A-B*	FRT^{101}	High	*a,c*
	P[ry+, hs-neo; FRT]11A	FRT11A	ND	*b*
	P[mini w+; FRT]18E-F	FRT$^{9-2}$	High	*a,c*
	P[ry+, hs-neo; FRT]18A	FRT18A	High	*b*
	P[ry+, hs-neo; FRT]19A	FRT19A	High	*b*
	P[ry+, hs-neo; FRT]19F	FRT19F	Low	*b*
2L	*P[ry+, hs-neo; FRT]29D*	FRT29D	ND	*b*
	P[ry+, hs-neo; FRT]34B	FRT34B	ND	*b*
	P[ry+, hs-neo; FRT]40A	FRT40A	High	*b*
2R	*P[mini w+; FRT]42B*	FRT^{2R-G13}	High	*a,c*
	P[ry+, hs-neo; FRT]42B	FRT42B	Low	*b*
	P[ry+, hs-neo; FRT]42C	FRT42C	Low	*b*
	P[ry+, hs-neo; FRT]42D	FRT42D	Medium	*b*
	P[ry+, hs-neo; FRT]43D	FRT43D	High	*b*
	P[ry+, hs-neo; FRT]50B	FRT50B	ND	*b*
3L	*P[ry+, hs-neo; FRT]69A*	FRT69A	ND	*b*
	P[ry+, hs-neo; FRT]72D	FRT72D	High	*b*
	P[mini w+; FRT]79D-F	FRT^{3L-2A}	High	*a,c*
	P[ry+, hs-neo; FRT]80B	FRT80B	Medium	*b*
3R	*P[ry+, hs-neo; FRT]82B*	FRT82B	High	*b*
	P[ry+, hs-neo; FRT]89B	FRT89B	ND	*b*
	P[ry+, hs-neo; FRT]93D	FRT93D	ND	*b*

ND = Not determined.
[a]Golic and Lindquist, 1989; [b]Xu and Rubin, 1993; [c]Chou and Perrimon, 1993 and 1996.

Table 3
Strains for Recombining Mutation onto FRT Arms

Chromosomes	Strains	Footnotes
X	*w P[mini-w+ hsπF]17B FRT18A*	*a*
	y w P[mini-w+ hsπM]5A, 10D FRT19A	*a*
	f^{36a} FRT19A; mwh kar^{2} ry^{506}	*a,b*
2L	*w; P[mini-w+ hsπM]36F FRT40A*	*a*
	y; P[y+ ry+]25F ck^{CH52} FRT40A/CyO; kar^{2} ry^{506}	*a,b*
2R	*w; FRT42D P[mini-w+, hsπM]45F*	*a*
	y; FRT42D pwn P[y+, ry+]44B/CyO; kar^{2} ry^{506}	*a,b*
	w; FRT43D P[mini-w+, hsπM]45F	*a*
3L	*y w; P[mini-w+ hsπM]75C FRT80B*	*a*
	yy; mwh (FRT73D?) FRT80B kar^{2} ry^{506}	*a,b*
3R	*w; FRT82B P[mini-w+ hsπM] 87E*	*a*

[a]Xu and Rubin, 1993; [b]Heitzler, P., unpublished.

Table 4
Strains for Adult Cuticular Clones

Chromosomes	Strains	Footnotes
X	*FRT18A; hsFLP3, MKRS/TM6B*	*a*
	FRT19A; hsFLP3, MKRS/TM6B	*a,b*
	y w FRT19A	*a,b*
	w sn^3 FRT19A	*a,b*
	f^{36a} FRT19A; mwh kar^2 ry^{506}	*a,d*
	Dp(3;Y;1)M2 y FRT19A/FM7; emc^{FX119} mwh kar^2 ry^{506}	*a,d*
	Dp(3;Y;1)M2 y M(1)o^{Sp} FRT19A/FM7; kar^2 ry^{506}	*a,d*
2L	*y w hsFLP1; P[y^+ ry^+]25F P[w^+ ry^+]30C FRT40A*	*a,b*
	y; P[y^+ ry^+]25F ck^{CH52} FRT40A/CyO; kar^2 ry^{506}	*a,d*
2R	*y w hsFLP1; FRT42D P[y^+, ry^+]44B P[w^+, ry^+]47A/CyO*	*a,b*
	y; FRT 42D pwn P[y^+, ry^+]44B/CyO; kar^2 ry^{506}	*a,d*
	y w; FRT42D P[mini-w^+, hsπM]45F M(2)S7/CyO; kar^2 ry^{506}	*a,d*
	y w hsFLP1; FRT43D P[w^+, ry^+]47A	*a,b*
	y w hsFLP1; FRT43D P[y^+, ry^+]44B	*a,b*
3L	*w hsFLP122; P[w^+]70C FRT80B*	*a,c*
	y w hsFLP122; P[ry^+ y^+]66E P[w^+]70C FRT80B	*a,c*
	y; mwh (FRT73D?) FRT80B kar^2 ry^{506}	*a,d*
	y; trc FRT80B kar^2 ry^{506}/TM6C ry^{CB} Sb Tb	*a,d*
	y w; jv P[ry^+ y^+]66E P[mini-w^+ hsπM]75C FRT80B kar^2 ry^{506}/TM3 ry^{RK} Sb	*a,d*
	y w; M(3)i^{55} P[mini-w^+ hsπM]75C FRT80B kar^2 ry^{506}/TM3 ry^{RK} Sb	*a,d*
3R	*y w hsFLP1; FRT82B P[w^+; ry^+]90E P[y^+ ry^+]96E*	*a,b*
	y w hsFLP1; FRT82B P[mini-w^+ hsπM]87E Sb^{63b} P[y^+ ry^+]96E	*a*
	FRT82B kar^2 ry^{506}	*a,d*
	pr pwn; FRT82B kar^2 ry^{506} bx^{34e} Dp(2;3)P32/FRT82B kar^2 ry^{506}	*a,d*

[a]Xu and Rubin, 1993; [b]Xu, T., et al., unpublished; [c]Ito, N., et al., unpublished; [d]Heitzler, P., unpublished.

Note that most eye clones marked with w^- will appear as dark or black patches against the background of a wild-type red eye. Only very large clones or clones located at the edge of the eye will appear white.

3.2. Induction of Germline Clones by FLP/FRT or X-rays

1. Set up crosses at 25°C such that progeny will be trans-heterozygous for a dominant female-sterile mutation (such as Ovo^{D1}) and the mutant gene or marker of interest (*see* **Notes 2**, **3**, and **8**).
2. Collect eggs for 24 h at 25°C.

3a. Heat-shock vials for 60 min in a 38°C water bath twice over a period of several days while progeny are in first and second larval instar stages. Adult virgin females collected from these crosses may be heat-shocked again before mating to initiate mitotic recombination in ovariole germline cells.

3b. X-ray twice, once during first and once during second larval instar stage. Place vials containing progeny close to X-ray source and expose to 1000R dose. Adult virgin females collected from these crosses may be X-rayed again before mating to initiate mitotic recombination in ovariole germline cells.

4. Allow females to recover at 25°C for a day before mating.

Table 5
Strains for Clones in Developing and Internal Tissues

Chromosomes	Strains	Footnotes
X	*w P[mini-w+ hsπM]5A, 10D FRT18A; hsFLP3, MKRS/TM6B*	[a]
	w P[mini-w+ hsNM]8A FRT18A	[a]
	w P[mini-w+ hsπF]17B FRT18A	[a]
	y w P[mini-w+ hsπM]5A, 10D FRT19A	[a]
	y w P[mini-w+ hsπM]5A, 10D M(1)o^{Sp} FRT19A/FM7	[a,c]
2L	*w hsFLP1; P[mini-w^+ hsπM]21C, 36F FRT40A*	[a]
	w hsFLP1; P[mini-w^+ hsNM]31E FRT40A	[a]
2R	*w hsFLP1; FRT42D P[mini-w^+, hsπM]45F/CyO*	[a]
	y w; FRT42D P[mini-w^+, hsπM]45F M(2)S7/CyO; kar^2 ry^{506}	[a,c]
	y w hsFLP1; FRT42D P[mini-w^+, hsNM]46F	[a]
	w hsFLP1; FRT43D P[mini-w^+, hsπM]45F,47F	[a]
	y w hsFLP1; FRT43D P[mini-w^+, hsNM]46F	[a]
3L	*y w hs FLP122; P[mini-w^+ hsπM]75C FRT80B*	[a,b]
	y w hsFLP1; P[mini-w^+ hsNM]67B (FRT73D?) FRT80B	[a]
	y w; jv P[ry^+ y^+]66E P[mini-w^+ hsπM]75C FRT80B kar^2 ry^{506}/TM3 ry^{RK} Sb	[a,c]
	y w; M(3)i^{55} P[mini-w^+ hsπM]75C FRT80B kar^2 ry^{506}/TM3 ry^{RK} Sb	[a,c]
3R	*w hsFLP1; FRT82B P[mini-w^+ hsπM]87E,97E*	[a]
	y w hsFLP1; FRT82B P[mini-w^+ hsNM]88C	[a]

[a]Xu and Rubin, 1993; [b]Ito, N., et al., unpublished; [c]Heitzler, P., unpublished.

Detailed protocols for dissection of imaginal disc tissues and staining of the π-myc and N-myc markers can be found in **refs. *3*** and ***13***.

Table 6
Strains for Generating Germline Clones

Chromosomes	Strains	Footnotes
X	*C(1)DX, y f/w ovo^{D1} v^{24} FRT^{101}/Y; hsFLP38*	[a,b]
	C(1)DX, y f/ovo^{D2} v^{24} $FRT^{9\text{-}2}$/Y; hsFLP38	[a,b]
2L	*P[mini w^+; ovo^{D1}]$^{2L\text{-}13X13}$ FRT40 A/S Sp Ms(2)M bw^D/CyO*	[a,c]
2R	*$FRT^{2R\text{-}G13}$ P[mini w^+; ovo^{D1}]$^{2R\text{-}32X9}$/S Sp Ms(2)M bw^D/CyO*	[a,b]
3L	*w; P[mini w^+; ovo^{D1}]$^{3L\text{-}2X48}$ $FRT^{3L\text{-}2A}$/ru h st βTub85D^D ss e^S/TM3, Sb*	[a,b]
3R	*w; FRT82B P[mini w^+; ovo^{D1}]$^{3R\text{-}C13a31\ n9}$/ru h st βTub85D^D ss e^S/TM3, Sb*	[a,c]

[a]Chou and Perrimon, 1993; 1996; [b]Golic and Lindquist, 1989; [c]Xu and Rubin, 1993.

4. Notes

1. The heat shock promoter is apparently not active in early embryo divisions. Workers wishing to produce clones in the embryo may need to use X-ray induction.
2. All crosses and egg collections should be carried out on well-yeasted rich medium such as the standard molasses-agar substrate.
3. It is important to culture flies at 25°C as heat shocking often kills larvae grown at 18°C.

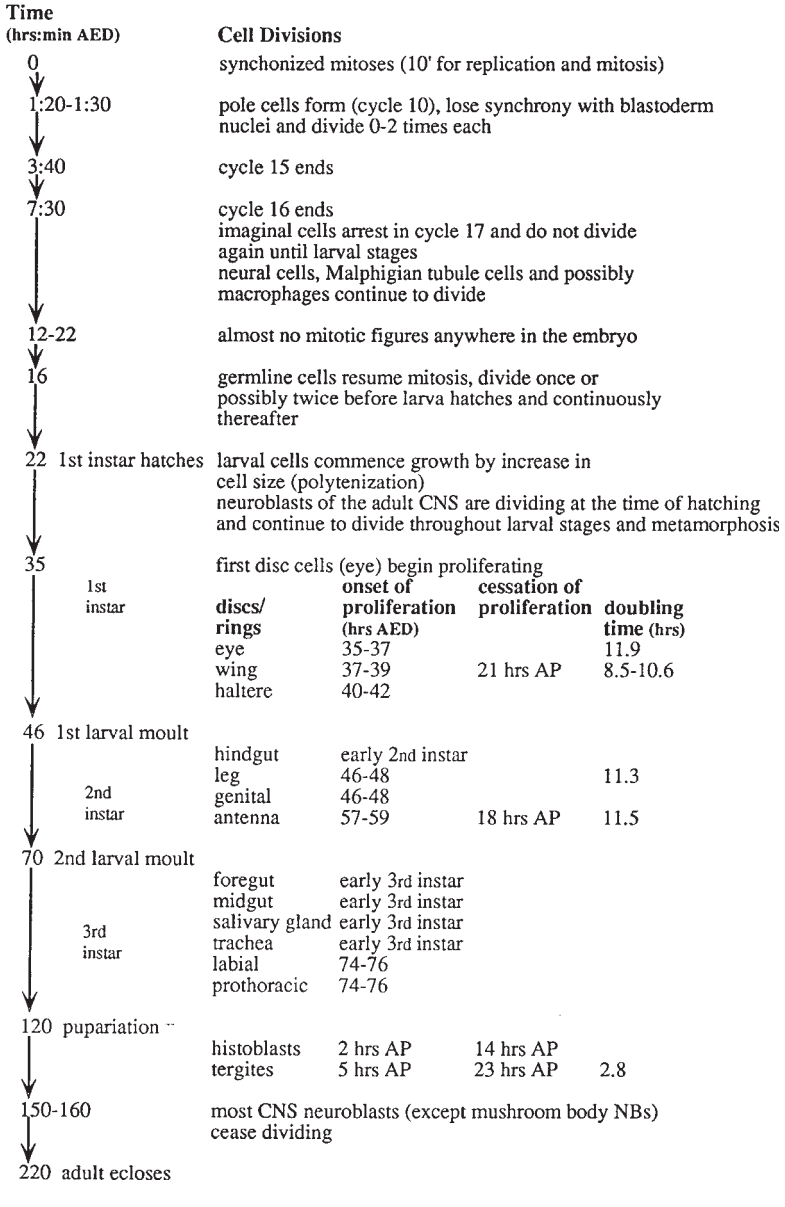

Fig. 3. Timeline of cell divisions in different tissues during *Drosophila* development. Times are given as hours after egg deposition (AED), except where noted. All times are for 25°C. Adapted from text and tables in 14–16. AP, after pupariation.

4. Crowded vials will produce divergent development rates among the progeny and thereby decrease the efficiency with which clones are produced at the precise desired developmental stage. If an experiment calls for large numbers of progeny, set up additional crosses in individual vials rather than crowd more females into a vial.
5. The production of clones using mitotic recombination is restricted to cells which are dividing at the time of heat shock (or X-ray). Thus, it is essential to induce FLP expression/ expose to X-rays when cells in the tissues of interest are actively dividing. Know the

developmental profile of the tissue(s) you wish to study (*see* **Fig. 3**). For Ey-FLP or GAL4/UAS-FLP, FLP is expressed and will get large clones.

6. When heat-shocking or X-raying older larvae or adult flies, push the cotton stopper down into the vial to restrict the animals' movement to as small a space as possible. Then ensure that this space is fully submerged (in the case of heat-shock) or placed very near the X-ray source; this will increase the frequency of clone production.
7. The temperature of the water bath for heat-shocking must be at 38°C. One degree less will dramatically decrease the clone frequency. On the other hand, temperatures higher than 40°C will kill the animals.
8. Remember that only a fraction of females collected from a germline clone experiment involving a dominant sterile mutation such as Ovo^{D1} will be fertile. It is useful to set up more than enough crosses to produce an excess of the required virgins, and to then be fastidious about maintaining a daily heat-shock (or X-ray) regimen and frequent collection of virgins.

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