A comparative study of mitotic delay and inhibition of transcription in cells exposed to UV and heat shock: the possible use of mitotic delay duration as a parameter for assaying extent of damage and recovery

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We have evaluated percentage inhibition of transcription and extent of mitotic delay induced by ultraviolet irradiation and heat shock, either applied alone or in combination, employing the synchronously mitotic plasmodia of the myxomycete *Physarum polycephalum* as a model system. While percentage inhibition of transcription, estimated soon after the perturbations, is a good indicator of the extent of a specific kind of damage, the duration of mitotic delay serves also as a reliable parameter for evaluating the extent of overall damage and rate of recovery of the system. Interestingly, according to this criterion, recovery from the effect of radiation is faster if it is preceded by a pulse of hyperthermia.

ULTRAVIOLET irradiation (UV) and heat shock (HS), two well-known cell-cycle perturbing agents, are known to induce significant mitotic delay in synchronously mitotic plasmodia of the myxomycete *Physarum polycephalum*[1-5]. Recently we analysed the synergistic effect of these two agents on mitotic-cycle duration, employing this organism as a model system[6]. We observed that the sequence in which the two agents were applied was crucial in determining the extent of mitotic delay: HS applied before irradiation (HSUV) reduced radiation-induced mitotic delay, whereas the reverse was the case when HS was applied after irradiation (UVHS). However, in that study, no strict correspondence could be established between mitotic delay and general protein synthesis inhibition in the two doubly perturbed systems. An apparent interference in total protein estimation by the preferentially synthesized heat-shock proteins in an otherwise protein synthesis-inhibited system could not be ruled out. This means that analysis made in terms of total protein synthesis would not be helpful in estimating cellular-level damage induced by the two perturbing agents. Therefore we have now analysed (Figure 1) the rate of transcription in these doubly perturbed systems, since, with UV alone, the mitotic delays are also accompanied by inhibition of macromolecular synthesis, including that of RNA[3]. Heat shock is also known to be a severe inhibitor of transcription in general; in a heat-shocked system, when the heat-shock loci (which number only a few) are turned on, all other active loci are turned off[7-9].

As expected, soon after the perturbations both doubly perturbed systems showed greater inhibition of transcription than the singly perturbed ones (Table 1). Of these, however, UVHS showed more inhibition than

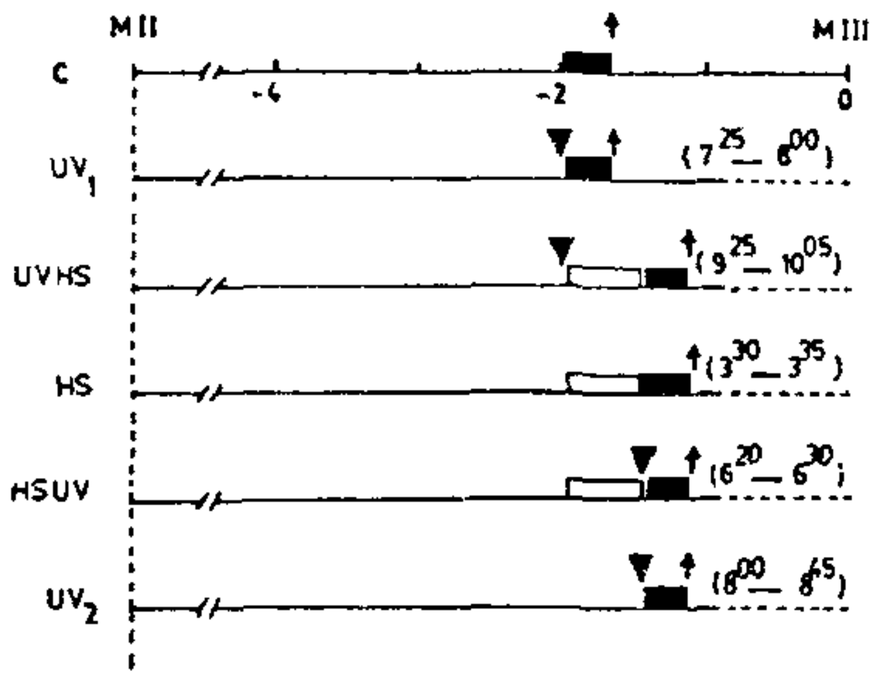

Figure 1. Schedule of UV and heat-shock treatments and [^{3}H]-uridine labelling with respect to the third post-fusion mitosis (PFM), MIII ('zero' point), of the control in different sectors of a plasmodium. Each plasmodium was cut into six sectors, which were used for the different treatments—C (control), UV1 and UV2 (irradiated), UVHS (irradiated and then heat-shocked), HS (heat-shocked), HSUV (heat-shocked and then irradiated). The numbers below the control line are times in hours prior to the 'zero' point (metaphase of MIII). MII, second PFM in the plasmodium as a whole. MIII in the treated sectors occur after a delay of several hours, indicated by a stippled line in each case. The duration between MII and MIII in control was ~12 h. *The range of delays obtained in each case in the different* experiments is given in parenthesis above the respective stippled line. ■ [^{3}H]-Uridine pulse; □, heat shock; ▼, UV irradiation; ⬆, time of collection of samples.

Methods. Suspension cultures of microplasmodia of *P. polycephalum* (M_3C strain), representing all stages of the mitotic cycle, were maintained on a semi-defined medium (SDM)[21] at 24 C. Mitotically synchronous sister surface (macro) plasmodia were made by coalescence of pooled microplasmodia on Whatman No. 40 filter paper[22]. Experiments were carried out during the G2 phase, within 2 h of the metaphase of the third PFM. Mitotic times were determined by observing ethanol-fixed smears in a phase microscope. Each plasmodium with the supporting filter paper was cut into six sectors, which were used for the different treatments as above. Since the mitotic delay due to UV increases from early to late G2 (ref. 3), and the duration of HS in our experiments was 30 min, it was necessary to carry out UV irradiation on two sectors, one at the beginning of HS (UV1) and another coinciding with the end of HS (UV2), such that the data from UV1 could be compared with UVHS and that from UV2 with HSUV. Heat-shock was given by transferring the sectors from their temperature of growth (24 C) to prewarmed (37°C) SDM-containing petri plates maintained in an incubator[5]. Shock was given for 30 min, after which the shocked sectors were transferred back to SDM at 24 C. As described earlier[3] UV irradiation was carried out at a dose rate of 10.26 J m^{-2} sec^{-1} using a Philips 15-W germicidal lamp emitting approximately 90% of the UV energy at 2537 Å. The total dose of irradiation was 1400 J m^{-2}. Immediately after the perturbations, the plasmodial sectors were pulse-labelled using [^{3}H]-uridine (80 μCi per ml of SDM, specific activity 16.5 Ci mmol^{-1} BARC, India) for 15 min. Incorporation was stopped by plunging the sectors in ice-cold water, followed by freezing by means of an ice-glycerine mixture (−6°C). Acid-soluble components were removed and acid-insoluble RNA was extracted from these sectors[23] and estimated by the method of Ceriotti[24]. For the assay of radioactivity in RNA, aliquots from the respective samples were spotted on Whatman GF/C filters and counted in an LKB RackBeta liquid scintillation counter using a toluene, PPO, POPOP cocktail.

Table 1. Inhibition of RNA synthesis* in UV-irradiated and heat-shocked G2-phase plasmodia of *Physarum polycephalum*

	Treatment*	RNA (cpm mg^{-1})	Inhibition with respect to control (%)
Plasmodium A (RNA in control = 176,840 cpm mg^{-1})	UV1	62,360	65
	UVHS	14,570	92
	HS	50,910	71
	HSUV	23,990	86
	UV2	51,950	71
Plasmodium B (RNA in control = 135,630 cpm mg^{-1})	UV1	61,590	55
	UVHS	11,360	92
	HS	47,220	65
	HSUV	22,180	84
	UV2	56,980	58

*See legend to Figure 1 for details.

the reverse combination HSUV. So, between these two systems, there is a direct correspondence between percentage inhibition of transcription and mitotic delay (Figure 1). Of the singly perturbed systems, HS showed equal or greater inhibition of transcription than UV1 or UV2 (Table 1), although the mitotic delay induced by heat shock alone is far less. For example, in comparable experiments, when heat-shock induced a delay in the range of 3–4 h during G2, UV produced delays in the range of 7–9 h. Though less striking, a similar observation can be made with respect to UV and HSUV. For example, mitotic delay in HSUV (~6 h 30 min, Figure 1) is less than that seen for the corresponding UV2 (~8 h 30 min, Figure 1)[6], although the percentage inhibition of RNA synthesis is more in the former (Table 1).

Since both UV and HS are known to induce severe repression of overall transcription, the extent of the repression has been taken as a parameter of a specific kind of perturbation, even though inhibition of transcription is induced by different mechanisms in these two cases. While the immediate repression of transcription by UV is apparently due to the lesions, particularly pyrimidine dimers, induced by it on chromosomal DNA[10], the profound conformational changes of chromatin induced by HS, probably as a result of change in DNA -protein interactions[11], are the cause of overall transcription inhibition in this case. In the latter system, the inhibition of overall transcription, however, is accompanied by the turning-on of a few specific loci, which code for heat-shock proteins (HSPs)[11].

The mitotic delay induced by either of these agents is a period during which cells recover from more than one kind of damage induced by these agents, and so we have taken it as a parameter to measure overall damage and recovery. For example, in addition to its effect on DNA and transcription, UV applied in late G2 (as we have done) inhibits translation[3], blocks the developing microtubule-organizing centres of the mitotic spindle[12,13], and reverses early-prophase nucleolar movements[1] in *P. polycephalum*. In mammalian cells about to enter mitosis, UV is known to induce synthesis of inhibitors of mitogenic factors, which then neutralize the latter[14]. Thus, during the mitotic delay period, in addition to the repair of the lesions on DNA[15], all these other damages must also be rectified. In the case of HS, the induced damages are of a less severe type, in that no lesions are produced on DNA itself, and the heat-induced denaturation of protein is made reversible to a great extent by the protection offered by the HSPs produced by the cells as a response to the heat shock, and so a faster recovery of this system is expected. The longer mitotic delay in the UV-irradiated system compared to the heat-shocked one, in spite of equal overall inhibition of transcription in both, must be viewed in the context of the points raised above.

Perhaps the most interesting aspect of this study is the protection offered to the plasmodia by a heat shock given prior to UV irradiation, as indicated by the shorter delay in the HSUV system, even though immediate inhibition of transcription here was more than that with UV alone. This is apparently due to the proposed protective effect of HSPs on macromolecules and organelles[11,16–19], some of which are known UV targets (nucleolus[1], chromosomes, and macromolecules in the process of synthesis[2,3]). As a consequence of this, the HSUV system recovers faster (than UV alone or UVHS), as indicated by the shorter delay. It is known that HSPs are produced as a response to stresses such as heat shock in the plasmodia of *P. polycephalum* as well, but not when exposed to UV[20]. Apparently then, the absence of the protection offered by HSPs at the time of irradiation is the reason for the longer delay seen in UVHS. In fact the delay in UVHS is even greater than that seen with UV alone.

Our studies show that, in the case of cycling cells, duration of mitotic delay can be taken as a useful and convenient parameter for comparing the extent of overall damage and rate of recovery with respect to perturbers having different modes of action on basic cellular processes, such as we have shown for UV and HS on transcription (present study) and translation[6].

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ACKNOWLEDGEMENTS. This work was supported by STEC, Government of Kerala Research Grant No. G.O.M.S.No.26/88 STED. We are grateful to Prof. E. Ramdaskuttan, Amala Cancer Research Centre, Thrissoor, for help in analysing the radioactive samples using their facility. We also thank P. R. Jayasree and T. Aravindan for help during the course of the experiments.

Received 16 February 1991; revised accepted 30 July 1991