Non-genetic individuality: chance in the single cell

John L. Spudich & D. E. Koshland, Jr

Department of Biochemistry, University of California, Berkeley, California 94720

The individuality of bacterial cells grown in homogeneous conditions was demonstrated by showing characteristic behavioural differences which persist over their lifespans. Poissonian fluctuation of small numbers of generator molecules can explain this individuality and may apply to such processes as differentiation and asynchrony of cultures.

BIOLOGICAL systems are constantly confronted with chance occurrences in their environmental conditions and internal processes. An elaborate biological apparatus has evolved to utilise chance in genetics for the survival of the species. Some feedback mechanisms, on the other hand, are designed to insulate the individual against chance fluctuations of environmental conditions. How much influence has chance in internal cellular processes in determining the metabolic state and behaviour of the single cell? More specifically, if cells containing identical chromosomes could be grown in environmentally homogeneous conditions, how similar would they be in their biochemical characteristics?

Uniform growth conditions can be obtained with agitated suspension cultures, since the spatial inhomogeneities which inevitably develop in stationary surface growth (for example, tissue culture plates or agar surfaces) are continuously randomised in swirled liquid culture. The problem of apparently non-genetic variation of cells in such a homogeneous environment has arisen in studies of several phenomena. For example, bacteriophage burst sizes[1], cell division times[2], bacterial flagellar phases[3,4] and β-galactosidase concentrations[5–7] all show variation difficult to attribute to environmental inhomogeneities or genetic causes. The question of individuality is difficult to pursue in these cases because one needs measures of characteristics of individual cells which can be ascertained essentially instantaneously and which do not perturb the cell's biochemical state. With such instantaneous measures an individual cell can be compared with itself at various stages of its life cycle and with other individual cells in an isogenic population. Such measures became available in our studies on chemotaxis and enabled us to approach the question of individuality in bacteria as an illustration.

The chemotactic migration of bacteria can be studied quantitatively. In the absence of chemotactic stimuli a bacterium shows brief periods of swimming in an approximately straight line (smooth swimming), interrupted by a rapid irregular motion, which reorients its swimming direction (tumbling). Tumbling is suppressed when the bacterium swims in a favourable direction in a chemical gradient and this regulation of tumbling frequency results in net migration up gradients of attractants and down gradients of repellents[8–10]. The modulation of tumbling frequency is caused by temporal changes in attractant or repellent concentration which are analysed by the sensing system and transmitted to the flagella[8,11]. For example, if the concentration of an attractant in the bacterial environment is suddenly increased, this temporal gradient transiently suppresses tumbling in the bacteria. Although the details of the temporal gradient sensing mechanism are unknown, control of tumbling can be rationalised as caused by changes in the levels of a tumble regulator[8,12]. The concentration of this tumble regulator is thus a key property of the cell since it provides an important survival mechanism for the bacterium. Its properties can be measured quantitatively in two ways: (1) in the sensitivity to stimuli as measured by recovery from temporal gradients[12,13] and (2) in the tumbling frequency when there is no gradient[9,12] as an indicator of the steady-state level of tumble regulator. These two properties were studied for individual bacteria as a test for non-genetic variability.

Sensitivity to gradients of attractant

Our first study of individual bacteria involved subjecting a bacterial clone of a tumbly mutant strain to a temporal gradient of serine, using the sudden mixing methods described earlier[8,13]. The transient smooth swimming in response to a temporal gradient stimulus is especially easy to quantify with tumbly mutants. The individual bacteria were kept in focus by rough tracking through a movable microscope stage which keeps the

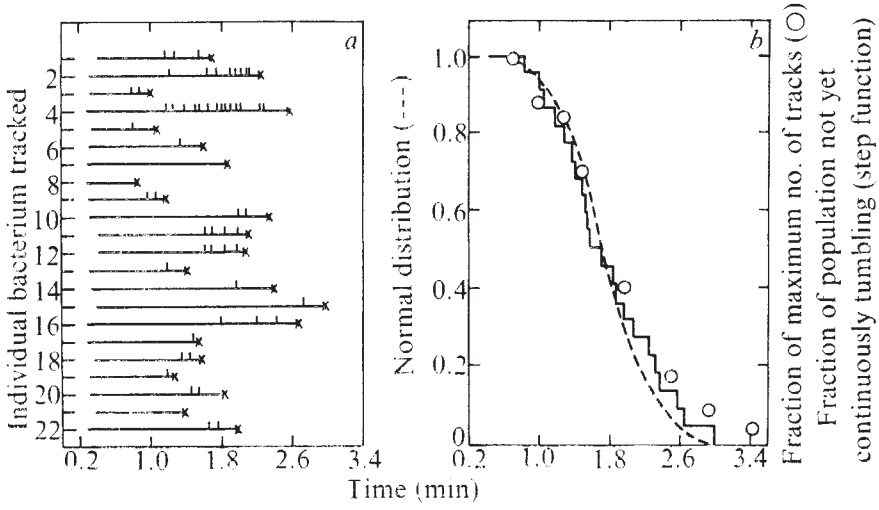

Fig. 1 Tumbly mutant recovery individuality. All bacteria were grown in homogeneous conditions as described previously[13]. In *a* the length of each line represents the time interval over which an individual bacterium was tracked. For each of the 22 lines the temporal gradient stimulus 0→0.5 mM L-serine was delivered to ST171 at time 0 (as previously described[13]) and the bacterium nearest the centre was followed until it tumbled continuously for 30 s. Times at which the bacterium briefly tumbles are are represented as ⊥, and the time at which it began to continuously tumble is represented as ×. From the data in (*a*) the fraction of the bacteria not yet continouusly tumbling was plotted against time to yield the step function in (*b*). ○, Tumble frequency assay[13] results for ST171 subjected to the same stimulus; – – –, a cumulative normal distribution with the same mean and variance as the data obtained from tracking.

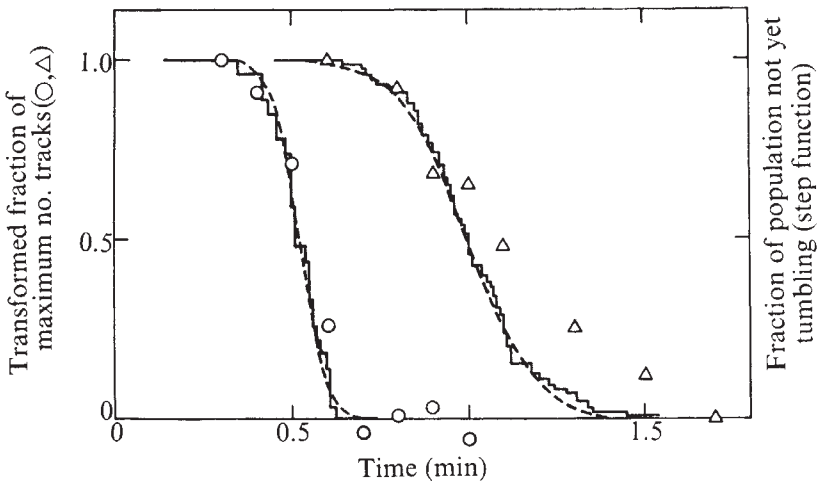

Fig. 2 Wild-type recovery individuality. Points are tumble frequency assay[13] results transformed by equation (1) (see text) for ST1 subjected at time 0 to the temporal gradient stimuli 0→0.01 mM (○) l-aspartate and 0→1.0 mM l-aspartate (△). Step functions are cumulative times to the first tumble after stimulation for these two same stimuli derived from tracking as described in Fig. 1. The dotted lines are cumulative normal distributions with the same means and variances as the two sets of tracking data.

bacterium in the centre of the field. A videotape recording could be played back in slow motion to describe in detail the behaviour over a long period. The results for 22 individuals of a constantly tumbling mutant subjected to a serine stimulus are shown in Fig. 1*a*. There is great variability in the time that individual bacteria take to return to their prestimulus tumbling pattern. Data of the same type (Fig. 2) were obtained for a wild-type strain which also swims smoothly immediately after stimulation and returns to intermittent tumbling (its normal non-gradient state). If data from tracking are plotted on the same graph as a recovery curve for a population subjected to the same stimulus in the tumble frequency assay[13], the curves are superimposable (Fig. 1*b*).

The results of Fig. 1*b* mean that the form of the recovery of a population from a stimulus is caused by the variability of the individuals in the population. In fact, if we define $F(t)$ as the fraction of a population which has recovered at time t after delivery of a stimulus and $S(t)$ as the probability of producing a smooth swimming track, equation (1) describes the behaviour of the bacteria. S_∞ is zero for the constantly tumbling mutant and has a finite value, greater than zero, for wild-type bacteria.

$$1-F(t) = \frac{S(t)-S_\infty}{1-S_\infty} \qquad (1)$$

The bacteria used in these experiments were at most 30 generations away from a single cell parent. An extremely high spontaneous mutation rate would have to be postulated to explain our data in terms of genetic variation, as the following calculation shows. Where m is the probability of occurrence of any mutation affecting chemotaxis per generation, after 30 generations the probability of a bacterium not containing a mutation is $(1-m)^{30}$. Therefore for at least 50% of the bacteria to have acquired one or more mutations affecting chemotaxis,

$$(1-m)^{30} < 0.5$$

or

$$m > 2.3\times10^{-2}$$

There are only about 10 genes specifically involved in *Salmonella* chemotaxis[14], but even the liberal estimate that a mutation in any one of 100 loci could alter chemotactic behaviour requires that the mutation rate per locus must be greater than 2×10^{-4}, which is at least 10^3 times higher than usual spontaneous mutation rates. Therefore we conclude that (1) there is a large non-genetic individual variability in the response of both tumbling mutants and wild-type bacteria to stimuli and (2) the results of the tumble frequency assay are a direct measurement of this variability.

Individuality and phase of cell cycle

The experiments described above establish that the cell types present do not show identical behaviour, but do not distinguish between the alternatives: (1) that the individuality is created by one or more random events in the history of the bacterium or (2) the cell has no intrinsic individuality but its properties vary according to its position in the cell division cycle. In the latter case the asynchrony of the culture would give the appearance of individuality where it does not in fact exist. To distinguish between these alternatives, the response of the bacteria were compared as a function of cell length. Cell length is known to be correlated with position in the division cycle[15] and bacterial lengths are clearly visible in the measurements from the tumble frequency assay. The lengths of smooth swimming tumbly mutant bacteria following stimuli at a time at which 95% of the bacteria were smooth swimming were compared with the same values when less than 10% of the bacteria were still smooth swimming. The former gives values for the overall population and the latter for the longest responding 10% of the bacteria. The distributions of the bacterial lengths are shown in Table 1. There is no significant shift in the distribution of the lengths as recovery proceeds and therefore the first alternative must be correct.

Repeated stimulation of a bacterium

If the different responses of isogenic bacteria are not related to the phase of the cell cycle, it can then be asked whether they (1) respond differently because of chance events during or

Table 1 Independence of recovery times and position in the cell division cycle

Body length (μm)	Fraction of population of body length indicated with > 95% smooth swimming	Fraction of population of body length indicated with < 10% smooth swimming
< 1.33	0.00	0.00
1.33–2.00	0.05	0.05
2.00–2.67	0.24	0.21
2.67–3.33	0.32	0.33
3.33–4.00	0.26	0.28
4.00–4.67	0.09	0.10
4.67–5.33	0.04	0.03
> 5.33	0.00	0.00

The lengths of ST171 bacterial bodies were measured on projected films from the tumble frequency assay[13]. 186 individual bacterial lengths were measured from photographs at times for which less than 5% of the bacteria had recovered from the serine stimuli to define the distribution designated >95% smooth swimming. 212 individual bacterial lengths were measured after more than 90% had recovered to establish the distribution in the last column.

Table 2 Response times (s) of individual bacteria on repetitive stimulation in growth conditions

Bacterium	*A*	*B*	*C*	*D*	*E*
Stimulus time (min)					
15	237	224	190	165	136
30	254	164	126	176	134
60	>300	265	191	136	145
75	>300	248	217	144	143
90	>300	253	172	152	148*
Average clockwise (smooth) response (s)	>278	231±18	179±15	155±7	141±3

Bacteria of strain SL3625, a leaky *fla AIII* mutant which produces small numbers of flagella, were tethered with flagella antibody to a coverslip by a modification of the procedure of Silverman and Simon[16]. Bacteria were grown as described[13] except 200 μM *p*-hydroxybenzoic acid (*p*HBA) was added to the growth medium to improve motility (unpublished results of J. Bar-tana, B. Howlett and D.E.K.). The coverslip with attached bacteria was used to cover an open temporal gradient observation cell of the type described by Macnab and Koshland[8]. The observation cell was previously filled with the wash medium to allow a liquid seal to form immediately the coverslip was put in place (time 0 min). The behaviour of bacteria in one microscopic field at ×500 magnification was recorded on videotape as follows. Cells were washed extensively (5 min at 12.7 chamber volumes per min) with the growth medium (without *p*HBA); at the times indicated the output line to the observation cell was switched to a line containing this same medium +10 mM α-methylaspartate and a 15-s pulse of 12.7 chamber volumes of attractant was delivered. The resulting immediate long clockwise rotation interval of an individual bacterium was measured as its "clockwise response". After 5 min, the line was switched back to medium not containing the attractant, and a 1-min pulse of 12.7 chamber volumes per minute delivered. Each bacterium in the field which was rotating for all stimuli was analysed by repetitive playback of the videotape. The doubling time of SL3625 in this medium is 112 min. Mean responses are reported ± 1 s.e.m.
*Bacterium *E* divided 20 s after the delivery of the stimulus at 90 min.

immediately preceding the time of the measurements, that is they are long responders at one time but short responders later, or (2) whether an individual bacterium is committed for an extended period to a certain type of response, that is some bacteria are short responders throughout their lifespan. To test the response of an individual bacterium over its cell division cycle, individual bacteria were tethered to the surface of microscope coverslips by antibodies to individual flagella, as described before[16]. Using this tethering technique, Berg and Tedesco[17] have shown that individual bacteria can be examined over extended periods and that tethered cells differ in their chemotactic behaviour. The tethering method permits attractant to be delivered and washed out at various intervals which are minutes or even hours apart and the response of the same individual bacterium recorded over the entire time span using videotape. Larsen *et al.*[18] established that counterclockwise rotation and clockwise rotation of a tethered cell (observed along the axis from the flagellum to the body) are equivalent to the tumbling and smooth swimming, respectively, of a free swimming cell.

Table 2 shows the results of repetitive stimulation of five tethered bacteria in the same microscopic field with the attractant α-methylaspartate. The attractant was delivered five times and removed completely between stimuli. The delivery of large stimuli of attractant suppressed counterclockwise (tumbling mode) rotation in each bacterium and caused a uniform clockwise (smooth swimming mode) rotation for several minutes with eventual recovery to intermittent clockwise and anticlockwise rotation. All bacteria were observed to increase to nearly double their mass during the measurements, yet the data show that their individual sensitivities to the stimulus were maintained. The same experiment was performed with six bacteria in a non-growth medium and these bacteria also maintained their tendencies to be either long or short responders to α-methylaspartate[19]. Thus, whether growing or not, individuals retain characteristically different sensitivity to stimuli for extended periods and the cells are not continuously randomising with respect to their chemotactic behaviour.

Relationship of unstimulated to stimulus-induced behaviour

If the bacteria differ in their characteristic recovery times, are they also different in some other feature of their sensory system? The unstimulated behaviour of tethered cells consists of alternating clockwise and anticlockwise rotation intervals with each of the two rotation modes terminated by Poissonian (random) processes[17]. The patterns of individual cells showed that the mean clockwise and counterclockwise intervals were not identical for all bacteria. In view of the randomness in lengths of clockwise and counterclockwise intervals for any individual bacterium, the theoretical standard error of the mean was calculated to compare with the empirical s.e.m. obtained from actual measurement of the clockwise and counterclockwise intervals (Table 3). The good agreement between theoretical and empirical values is assurance that the amount of data collected to determine the intervals is sufficient.

On comparison of these spontaneous rotation intervals with the response to stimuli (Table 3) an excellent correlation was observed. The size of the clockwise response to an α-methylaspartate stimulus for an individual bacterium depends nearly linearly on its mean clockwise (smooth swimming mode) interval in non-stimulus conditions as shown in Fig. 3. The size of the anticlockwise interval (tumbly mode) does not seem to influence the stimulus behaviour.

To check this in another way, 82 free swimming cells were examined both for the time to the first tumble after temporal gradient stimulation with aspartate and for the mean time between tumbles for 1 min after the first tumble. Although this measurement is less precise than measurements with tethered cells (because a particular individual can be observed for only one stimulation and for only a short time after the stimulus), we found a correlation coefficient of 0.5 between the average length of a run and the recovery time.

These data provide a correlation between the steady-state tumbling pattern of individual bacteria and their sensitivity and recovery from stimuli. The steady-state pattern of tumbling,

Table 3 Unstimulated behaviour and smooth responses of individual bacteria

Bacterium	*F*	*G*	*H*	*I*	*J*
No. of intervals	154	84	186	134	82
Mean clockwise (smooth rotation mode) length (s)	2.45	1.93	1.40	1.54	2.97
Measured s.e.m.	0.28	0.26	0.16	0.19	0.54
Calculated s.e.m.	0.28	0.30	0.15	0.19	0.46
Mean anticlockwise (tumbly rotation mode) length (s)	0.88	1.69	0.80	1.63	0.70
Measured s.e.m.	0.12	0.25	0.08	0.22	0.16
Calculated s.e.m.	0.10	0.26	0.08	0.20	0.11
Mean clockwise (smooth) response (s):	211±10	152±6	120±8	123±6	247±7

The experiment was performed by a procedure identical to that of Table 2 except: (1) instead of growth medium cells were washed with Vogel Bonner salts + 10^{-5}M EDTA; (2) attractant (10 mM α-methyl aspartate) was prepared in Vogel Bonner salts + 10^{-5}M EDTA; (3) after the initial wash of 5 min of 12.7 chamber volumes per min, a continuous flow rate of 1.6 chamber volumes per min was maintained throughout the experiment; (4) at 5 min intervals the input line was switched between attractant and buffer solutions to give a total of six repetitive stimuli. About 2 min of video recordings of the bacteria in periods immediately preceding delivery of attractant were examined in slow motion (sevenfold) replays. Keyboard inputs to a Data Span 500 paper tape punch with timing circuitry[29] were punched when the bacterium being analysed reversed rotation direction for at least 1/4 of a full turn. The paper tape containing the reversal information and the automatically entered times of keyboard entries was processed by computer. The calculated s.e.m. was determined with the assumptions that (1) the measured mean is the true mean and (2) both clockwise and anticlockwise intervals are distributed exponentially[17]. Clockwise responses are reported as the mean ± 1 s.e.m.

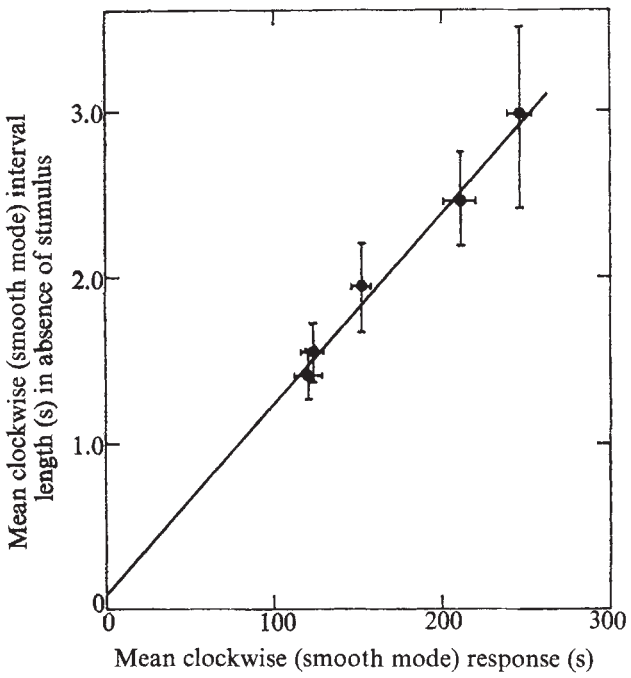

Fig. 3 Unstimulated clockwise (smooth mode) rotation related to stimulus induced clockwise (smooth mode) rotation. The prestimulus clockwise (CW) mean interval length and the mean CW response were taken from Table 3. Error bars represent ± one standard error of the mean. The line is a least squares fit to the data.

therefore, is also a feature of the bacterial individuality and is retained throughout the generation time of the bacterium.

Implications of individuality

Study of individual bacteria in various ways has established that a bacterial culture which is genetically homogeneous and grown in homogeneous nutrient conditions nevertheless produces characteristically different individuals. These individuals retain their properties throughout their life cycle, showing that the individuality is not a momentary effect of random chance bombardments by a fluctuating environment. Moreover, the individuality is not a function of position in the cell division cycle.

How could such individuality be produced? A bacterium is a small cell. In the process of division and replication of the cellular components there may well be certain molecules which are present in such low amounts that they are subject to Poissonian variation. In general, the standard deviation of a Poisson distribution is the square root of n, where n is the mean number of events. If there are 10^4 molecules of a particular type, the s.d. would be 100 molecules, giving a 1% deviation. If, on the other hand, only 100 molecules are produced, a 10% s.d. would arise. In many cellular processes less than 100 molecules participate (for example, there are only 10–20 molecules of *lac* repressor per cell[20] and enzyme cofactor concentrations are extremely low[21]). In other processes there may be more total molecules but their ultimate number may have been determined by a small number of generating molecules (for example, there are only 6–14 *trp* mRNA molecules per bacterial cell, and each mRNA molecule is translated an average of 20 times[22]). Probabilistic fluctuations in small numbers of molecules have been discussed as possible causes of bacteriophage burst size variation by Delbrück[1] and bacterial β-galactosidase concentration variation by several investigators[5–7].

In the case of chemotaxis, it has been postulated that the tumble regulator is produced and destroyed according to scheme 2, implying that some enzymes or structural molecules control the velocities of the steps represented by v_f and v_d. If a small number of mRNA molecules code for the enzyme which, for example, controls the step v_d, Poissonian fluctuation in the mRNA concentration would generate quite different total numbers of molecules of this enzyme in different individuals. If this affects not only the steady-state tumbling pattern, but also the rate of recovery from a stimulus[12], both properties would be different from individual to individual over the lifespan of the cells. Conversely, if a particular cellular component were produced in a much larger number of copies

$$W \xrightarrow{v_f} X \xrightarrow{v_d} Y \qquad (2)$$

where X = tumble regulator over a much longer interval, Poissonian variation would be relatively small and one would not expect much variation in this property from cell to cell. The number of copies of an individual cellular component, at some crucial period in the life of the cell, would therefore determine whether or not that component would contribute to the non-genetic variability of the population.

The existence of such variability leads one to ask whether it has some advantage to the organism or whether it is a disadvantage which must be surmounted in the development of the species. Although there may be no significant selective pressure against non-genetic variability, one can certainly make an argument that non-genetic variability aids in the survival of a population subjected to widely varying conditions during its lifetime. If the bacterium has a single or monolithic reaction to a particular chemical gradient, it might migrate as a colony into a toxic situation or fail as a colony to be sufficiently sensitive to an essential new nutrient. If there were individual bacterial variation in a genetically homogeneous population, a few fractions of the bacteria might either be supersensitive or insensitive. These bacteria would normally not survive as effectively as the bulk of the more average optimised population. They would then have lower survival probabilities in most circumstances, an unimportant loss if the main body of the colony survived. If, on the other hand, the toxic and lethal situations described above arose, a small fraction, on the wings of the probability distribution, might constitute the only bacteria to survive and would then reproduce the species for future generations. Genetic variation would not accomplish the same purpose, since selection in a rare toxic situation would produce a mutant poorly adapted to the more common conditions of the environment. The population, however, was selected over evolutionary time for survival in all of the widely varying conditions of the environment. Thus non-genetic variability would be a preferred mechanism for accommodation to random fluctuations in the environment and genetic variability the preferred mechanism for accommodation to long lasting environmental changes. Induction and repression of enzyme synthesis reflect intermediate accommodations to fluctuations of a lengthy but impermanent type.

It would be intriguing if certain properties were found to be insulated against chance by the large number of copies in the cell at all crucial stages and other properties deliberately to originate in a small number of starting molecules, to create non-genetic diversity. An interesting example may arise in the differentiation of individual *Dictyostelium* amoebae into multicellular fruiting bodies. This development depends on the existence of a small number of early cyclic AMP-emitting founder cells which are believed to be genetically identical to the other cells in the population which are not early emitters[23]. The difference between a founder cell and other cells in the culture seems to be the result of molecular accidents in the cell's individual history and this could be explained by a Poissonian distribution of properties deriving from small numbers of a cellular component as discussed here. Possibly over evolutionary time, larger cells and larger individuals were selected for decreased non-genetic variability; for example, identical human twins are morphologically much more similar than twin bacteria, and in higher species the genetic variability

carries the main load of ensuring the survival of the species. Poissonian variation at early stages in differentiation may be an advantage for the species even though ultimately chance is eliminated because of large numbers of cells developed in the individual.

Poissonian variation may be the reason for the rapid asynchronisation of synchronised prokaryotic[21] and eukaryotic[25] cell cultures. If one or more steps leading to growth and cell division are controlled by a small number of molecules, then a Poissonian distribution should exist and cell cycles would quickly get out of phase. In fact there is evidence for individuality in bacterial division times[2] of the type we observe in chemotactic behaviour. Also in eukaryotic cells synchrony is not maintained even in populations selected for genetic homogeneity[25]. To explain this, Smith and Martin[26] have proposed that growth of cultured cells is dependent on a random event occurring with a probability which is constant for all individual cells in postulated "indeterminate" phase in G_1 (the "A-state"). As evidence for this hypothesis they present generation time distributions which are exponential over most of their range. A chance external event could explain the probabilistic transition out of the A-state, if the Smith and Martin model is correct. Alternatively, as suggested here, the number of generating molecules for an enzyme required for passage through G_1 could be so small that Poissonian fluctuations result in a broad distribution of concentrations of this enzyme among the cells. Such a non-genetic individuality would be consistent with rapid asynchronisation, correlation of sibling generation times[2,27,28] (if the enzyme half life is longer than one generation time), and the exponential character of the distributions of Smith and Martin. If Poissonian variation determines rates of passage through G_1, or any stage in the cell cycle, cell synchrony over extended periods will be difficult to attain. If our explanation is correct, a possible approach to obtaining a synchronous cell line would be genetic manipulation, for example by episomes, to increase significantly the concentrations of the rate limiting enzymes.

Biological systems therefore can produce individual non-genetic diversity through a few generating molecules which determine a property at some key stages of development or can ensure against such chance diversity by increasing the number of generating molecules to a size which eliminates significant internal variation.

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letters to nature

Observations of X-ray and γ bursts

We report on some bursts of hard X rays seen in March 1976 by the Ariel-V scintillation telescope (ST). The timescale of a few seconds, the correlation with low energy events seen by a proportional counter on the same satellite (ref. 1 and S. J. Bell-Burnell, private communication) and a photon energy > 50 keV, are factors which suggest a connection in origin between the gamma bursts of Klebesadel *et al.*[2] and the X-ray events noticed by Grindlay *et al.*[3]. A delayed arrival at high energies seems to be an important feature of our observations.

The Ariel-V scintillation telescope (area 8 cm^2, 11° effective FWHM field of view) observed in a direction centred on RA 17 h 27 min, dec. −33.5° (1950) during March 8–11 with a broad energy channel extending from 50 to 190 keV. Counts from 0.5-s intervals were stored sequentially in sets (bins) of 16 locations which were scanned four times before proceeding to the next set. Each bin thus contained the superposition of 4 time profiles, 8 s long. An identical data mode was used for the 2–7-keV collimated proportional counter (CPC) of ref. 1. Burst sources MXB 1730–335, MXB1728−34, MXB1743−293 and MXB1742−297 were in the field of view of the ST but only the first two could be seen by the CPC. MXB1730−335 is the repetitive burster with small events as close as 7 s apart, while the others give at the most ~5 bursts d^{-1} (ref. 4).

Four very important proportional counter bursts were selected corresponding to those times when the ST data was expected to be free of all spurious events (events A, C, E, F Table 1), two of which (E, F) are plotted in Fig. 1, together with data from the ST. Absolute time is known to ±30 s and this is shown at the beginning of each 32-s bin. In two cases the CPC data showed a pulse at least 8 s long; the double peaks probably arise from two successive scans. In at least 3 cases, the ST increase appeared delayed with respect to the CPC data. Because of the superposition of scans the delay cannot be determined uniquely. Table 1 lists the limits within which the delays lie, the significance in s. d. (σ) of the ST events, the pulse durations and the total energy in erg cm^{-2} assuming all high energy photons were at 50 keV.

In the case of events A and F, the larger of the two figures for minimum delay is more likely; the smaller figure results from taking smaller, secondary peaks in the CPC data as the associated events. Note the tendency for the high energy channel to carry at least as much energy as the low energy channel. Suggestions have been made for 1.39- and 1.36-h periodicities in the intervals between some bursts from this region (ref. 1 and Carpenter *et al.*, to be published). Only event A fits such a scheme (with a 1.36-h period).

To relate our work to searches for γ bursts, a scan of all the suitable March 8–11 data was made to pick out statistically